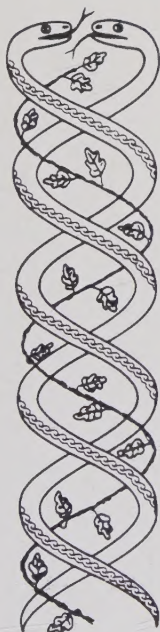


HEALING OF SMALL INTESTINAL ANASTOMOSES

**An experimental evaluation
of mechanical properties
and collagen changes**

Kent Jönsson



Malmö 1985

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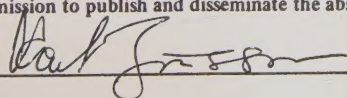
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Title and subtitle HEALING OF SMALL INTESTINAL ANASTOMOSES. An experimental evaluation of mechanical properties and collagen changes.			
Abstract The purpose of the present study was to investigate the strength development of small intestinal anastomoses and analyse changes in collagen and non-collagenous substances in relation to anastomotic strength and to compare healing in the small and the large intestines. Anastomoses were performed in a standardized way in jejunum, ileum and left colon and ileocolic anastomoses were performed after right hemicolectomy. Anastomotic complications were registered. Breaking strength was determined with and without sutures. Standardized biopsies were taken for analysis of dry weight, collagen (content, concentration and synthesis) and protein synthesis. Collagen was determined as hydroxyproline. Synthesis of collagen and protein was studied by in vivo incorporation ₃ of ³ H-proline, using a pulse labelling technique and determination of ³ H-hydroxyproline and total radioactivity. Anastomotic strength successively decreased during the first three post-operative days to approximately 15% of the immediate postoperative value. During this phase loss of collagen could not be detected. From day four on, a rapid increase in strength was recorded. This could be related not only to deposition of collagen in the tissue bridging the anastomosis but also to deposition of collagen in the intestinal wall leading to increased suture holding capacity. After fourteen days sutures do not contribute to anastomotic strength. Collagen and non-collagenous substances increased earlier and more markedly after left colonic anastomoses than after ileal anastomoses. Studies on ileocolic anastomoses indicate that the bacterial count in the intestinal content influences collagen metabolism in the ileal wall and anastomotic strength. Changes in the intestinal wall nearby an anastomosis are of major importance for anastomotic integrity.			
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HEALING OF SMALL INTESTINAL ANASTOMOSES

An experimental evaluation of mechanical properties
and collagen changes

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HEALING OF SMALL INTESTINAL ANASTOMOSES

An experimental evaluation of mechanical properties
and collagen changes

Kent Jönsson



Malmö 1985

To

Ega, Nina, Cara and Aira

This thesis is based on the following papers, which are referred to by their Roman numerals.

- I BREAKING STRENGTH OF SMALL INTESTINAL ANASTOMOSES.
Jönsson K, Jiborn H, Zederfeldt B.
Am J Surg 1983; 145:800-803.
- II CHANGES IN COLLAGEN CONTENT OF THE SMALL INTESTINAL
WALL FOLLOWING ANASTOMOSIS.
Jönsson K, Jiborn H, Zederfeldt B.
Accepted for publication in Am J Surg 1984.
- III COLLAGEN METABOLISM IN SMALL INTESTINAL ANASTOMOSIS.
Jönsson K, Jiborn H, Zederfeldt B.
Submitted for publication.
- IV MECHANICAL AND BIOCHEMICAL ALTERATIONS IN THE INTESTI-
NAL WALL ADJACENT TO AN ANASTOMOSIS.
Jönsson K, Jiborn H, Zederfeldt B.
Accepted for publication in Am J Surg 1984.
- V COMPARISON OF HEALING IN THE LEFT COLON AND ILEUM.
Changes in collagen content and collagen synthesis in
the intestinal wall after ileal and colonic anastomoses
in the rat.
Jönsson K, Jiborn H, Zederfeldt B.
Accepted for publication in Acta Chir Scand 1985.
- VI HEALING OF ILEOCOLIC ANASTOMOSES. Breaking strength and
collagen content in the intestinal wall after ileocolic
resection and anastomosis in the rat.
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HEALING OF SMALL INTESTINAL ANASTOMOSES

An experimental evaluation of mechanical properties and collagen changes

INTRODUCTION

Performance of an anastomosis is a part of many surgical procedures involving the gastrointestinal tract. Leakage and dehiscence of such anastomoses are serious complications with high morbidity and mortality. (For review see Tagart 1981). Several clinical and experimental studies on the etiology of anastomotic leaks in the stomach and the colon have been carried out, but there appear to be few that deal with the small intestine which is surprising. This could be explained by the clinical impression that small intestine anastomoses become complicated less often than, for example, those in the colon.

Statistics over the number of anastomoses performed in Sweden in the different parts of the intestine are available for 1981. In that year about 2800 anastomoses involving the stomach and about 4000 in the colon were performed. In the small intestine more than 1000 end to end anastomoses and another 1000 operations involving closure of intestinal defects were performed (Socialstyrelsen 1981).

The frequency of anastomotic complications varies markedly in clinical reports, e.g. between 2 and 18% after anastomoses in the large intestine (Debas & Thomson 1972; Morgenstern et al. 1972; Schrock et al. 1973; Matheson & Irving 1975; Everett 1975; Wilson & Beahrs 1976; Goligher et al. 1977; Fielding et al. 1980; Tagart 1981) and between 5 and 21% after anastomoses involving the oesophagus (Gunnlaugsson et al. 1970; Inberg et al. 1971; Hermreck et al. 1976; Alfonso et al. 1977; Papacristou et al. 1979; Wilson et al. 1982; Kirkpatrick & Siegel 1984). There are few reports on the leakage rate of anastomoses in the small intestine but

clinically the impression is that this rate is much lower than in anastomoses in the large intestine. On the other hand, in a clinical study comprising both large and small bowel anastomoses Irving & Goligher (1973) reported approximately the same incidence of anastomotic leaks in the different parts of the intestine located within the peritoneal cavity. In that study, however, the patients with small bowel anastomoses were relatively few. Anastomotic leakage after small intestinal operations for morbid obesity has been found to vary between 1 and 4% in several studies (Bray 1977; Alden 1977; Griffen et al. 1977; Butler et al. 1980; McFarland et al. 1985). It should be noted, however, that in these studies only the most serious leaks were registered.

One reason for the large variations in the reported incidence of anastomotic complications may be the method used for diagnosis. Many reports are based on clinically important leaks only. However, when special methods are used to detect leakage e.g. early radiological examination after colonic surgery, leakage has been found in a much higher frequency, e.g. in colorectal anastomosis over 50% (Goligher et al. 1970) and more recently 33-35% (Goligher et al. 1977; Tagart 1981). There are several other reasons for variations in complication rate such as anatomic localization of the anastomosis and the skill of the surgeon. In a large multicentre study it was found that the single most important factor for the outcome of the operation was the surgeon in charge (Fielding et al. 1980).

It is also well known that any kind of bowel damage has a higher rate of complication following surgery, e.g. in irradiated small bowel a dehiscence rate of 36-44% accompanied by a mortality of 13-21% has been reported (de Cosse et al. 1969; Sugg et al. 1963; Swan et al. 1976; Galland et al. 1978). The operative mortality is about 5% after simple obstruction (Shatila et al. 1976; Stewardson et al. 1978; Ellis 1982) but 15-30% after strangulation (Barnett et al. 1976; Bizer et al. 1981; Ellis 1982).

It is thus difficult to assess the complication rate in anastomoses in different parts of the gastrointestinal tract but the frequency is, no doubt, high in all parts including the small intestine.

Dehiscence and leakage may be due to

- defective sutures
- insufficient strength of the sutured intestinal wall
- defects in healing.

Modern suture materials used for intestinal anastomoses are strong and retain their strength for an adequate time. Therefore nowadays suture defects should be of small consequence provided that a correct tying technique is used (Tera & Åberg 1976a; Holmlund 1977).

The strength of the intestinal wall and thus the tissue encircled by the suture is of importance for the suture holding capacity. It is known that tissue will tear when mechanical tests of anastomoses are performed (Howes 1940). Loss of anastomotic strength during the first days after surgery has repeatedly been shown (Chlumsky 1899; Cronin et al. 1968a; Wise et al. 1975; Hesp et al. 1984a; Blomquist et al. 1984a). It has been suggested that this loss is due to collagen breakdown in the intestinal wall caused by collagenolytic enzymes (Hawley 1969; Högström et al. 1985a).

The rate of healing is influenced by several factors, both general and local. General factors such as age (Debas & Thomson 1972; Schrock et al. 1973; Irvin & Goligher 1973; Tagart 1981), hypovolemia (Zederfeldt 1957), hypoxia (Niinikoski 1969; Hunt et al. 1972), metabolic disorders, e.g. uncontrolled diabetes mellitus (Goodson et al. 1977; Gottrup & Andreassen 1981) uremia (Colin et al. 1979; Goodson et al. 1982) malnutrition (Daly et al. 1972; Irvin & Goligher 1973; Irvin & Hunt 1974) and medication, e.g. glucocorticoids (Ehrlich & Hunt 1968; Gottrup & Oxlund 1981), are known to affect the healing process adversely. Important impairing local factors are infection (Yamakawa et al. 1971; Hawley

1973; Schrock et al. 1973; de Haan et al. 1974; Irvin 1976; Bucknall 1980; Hesp et al. 1984b), obstruction in the colon (Dutton et al. 1976; Irvin & Greaney 1977; Kelley et al. 1981; Öhman 1982; Wolmark et al. 1983) as well as in the small intestine (Barnett et al. 1976; Stewardson et al. 1978; Bizer et al. 1981; Ellis 1982;), inadequate mechanical preparation with faecal loading (Irvin & Goligher 1973; Smith et al. 1983;) and irradiation (de Cosse et al. 1969; Schrock et al. 1973; Swan et al. 1976; Galland et al. 1978; Murphy et al. 1980). Another fundamental local factor is the technique used for performing the anastomosis. Inverting, serosa to serosa, suture technique is preferable (Hargreaves & Keddie 1968; Trueblood et al. 1969; Irvin & Edwards 1973) to the everting suture technique (Getzen & Holloway 1966). Single layer technique may be better than double layer (Gambie 1956; Buchin & van Geertruyden 1960; Irvin & Edwards 1973; Matheson & Irving 1975; Everett 1975), but this is not the opinion of all investigators (McAdams et al. 1970; Irvin et al. 1973; Goligher et al. 1977; Fielding et al. 1980). Interrupted sutures have in experimental studies been demonstrated to be superior to continuous suture (Hawley 1969; Jiborn et al. 1978a, 1980b). It has also been shown that drains are detrimental to anastomotic healing (Berliner et al. 1967, Manz et al. 1970; Duthie 1972; Crowson & Wilson 1973, Ravitch et al. 1981; Smith et al. 1982).

It is obvious that several factors and different mechanisms may cause dehiscence and leakage of intestinal anastomoses. Our knowledge is incomplete especially as regards the small intestine which has been studied much less than, for example, the colon. It is not known if there are differences in healing between the colon and the small intestine. Comparison of healing in the small and large intestines have given conflicting results (Wise et al. 1975; Hesp et al. 1984a). The anatomical structure as well as the blood flow vary in different parts of the intestine. This is also the case for the consistency of the intestinal contents and the bacterial flora. Thus, the non-uniformity of the intestine calls for separate studies of its various parts and findings in the colon cannot be regarded valid for the small intestine.

The purpose of the present study was to:

- investigate the strength development of anastomoses of the small intestine
- analyse the importance of sutures for anastomotic strength at different times after the operation
- analyse changes in collagen and non-collagenous substances in the intestinal wall around an anastomosis
- relate the biochemical changes to strength development
- compare the early course of healing in the small and large intestines.

MATERIAL AND METHODS

Experimental animals

Wistar male rats (Møllegaards Breeding Center Ltd, Denmark) weighing 200-300 g were used. The animals had free access to standard laboratory food and water before and after the operation. Weights were recorded at the time of operation and after sacrifice.

Anaesthesia and operative technique

The animals were anaesthetized by intraperitoneal injection of chloral hydrate (36 mg per 100 g body weight). After shaving the abdomen it was opened with a midline incision. An anastomosis was performed after resection of a 1 cm long segment either in the jejunum 5-8 cm from the pyloric region (I and II), in the ileum 5-8 cm from the ileocecal valve (I to V) or in the left colon 3 cm above the peritoneal reflection (V). In study VI a right hemicolectomy including 5 cm of the ileum was performed with an anastomosis between ileum and colon at the major flexure (Lindström et al. 1979). The anastomoses were made of one layer interrupted, inverting sutures of 7-0 polypropylene (Surgilene^R) in all studies except IV, where 7-0 silk was used. Stay sutures were used to facilitate standardization of anastomoses. The stitches were inserted through all layers except the mucosa at a distance of approximately 1 mm from the transection line. For anastomoses in the small intestine and the left colon 14-16 sutures were used and for ileocolic anastomoses 16-17 sutures

res. The anastomoses were carried out with the help of a magnifier. Fascia and skin were closed separately with continuous 4-0 polyester sutures (Tycron^R).

Gross appearance and anastomotic diameter

The presence of abscesses, anastomotic dehiscence and granulomas was carefully noted and animals with these complications were excluded from further study. The anastomotic diameter was determined with the help of a caliper after clamping the specimens in the tensiometer (see below). Specimens wider than 8.7 mm in the small intestinal anastomoses and 9.5 mm in the ileocolic anastomoses were excluded.

Determination of mechanical strength

An approximately 6 cm long segment with the anastomosis in the middle was freed from adhesions and excised. This segment was used for breaking strength determinations on a specially constructed motordriven tensiometer which provided an increasing force from 0.03 to 0.05 N/s in the interval from 0 to 3 N. The specimen was mounted with the anastomosis midway between two clamps 1 cm apart. Sutures were left in place, except in some tests (I) when on day 7 and day 14 sutures were carefully removed under a dissecting microscope.

Collagen studies

I. Determination of specimen dry weight, collagen content and collagen concentration

Jejunum, ileum, ileocolic and left colon anastomoses were analysed. In all studies except IV, three consecutive 1 cm long segments were cut out on each side of the anastomoses. In study IV three 0.5 cm segments were analysed of which the middle segment contained the anastomosis in the centre.

All samples were scraped with a pair of scissors to remove intestinal content and mucus and dried to constant weight in an oven at 100⁰ C. Dry weight was recorded and the segments were hydrolyzed in 6 N HCl for six hours at 130⁰ C. Hydroxyproline (hypro) was determined according to Stegemann & Stalder (1967).

Tissue dry weight was expressed in mg/segment.

Hydroxyproline content was expressed as μg hypro/segment.

Hydroxyproline concentration was expressed as μg hypro/mg dry weight.

II Determination of collagen and protein synthesis

The synthesis of collagen and other proteins was studied by in vivo incorporation of radioactive proline. The animals were given 3.7 MBq (100 μCi) (III) or 7.4 MBq (200 μCi) (V) L-proline (2-3-4-5- ^3H) (New England Nuclear, specific activity $3.7\text{--}4.4 \times 10^6$ MBq/mmol) intravenously 24 h prior to sacrifice. The incorporation of ^3H -proline into proteins and collagen was determined according to Juva & Prockop (1966) as applied to intestinal healing by Jiborn et al. (1980a). The radioactivity was measured in a liquid scintillation counter (LKB Wallac 81000). At least 1000 cpm over background were registered.

The following calculations and terms are used:

Specific activity of collagen (dpm ^3H -hypro/ μmol hypro) as a measure of newly synthesized collagen in relation to the total amount of collagen in each specimen.

Total radioactivity (total dpm ^3H /segment) as a measure of the synthesis of all proteins containing proline including collagen.

Statistical methods

Mean, standard deviation and standard error of the mean were calculated. Comparisons of means were carried out by Student's t-test for unpaired observations. Student's t-test for paired observations was used for comparisons of results obtained from one animal. By means of the Chi-square test analysis of complication rate was carried out.

COMMENTS ON MATERIAL AND METHODS

Experimental animals

Rats were chosen as experimental animals mainly because this is the species usually selected for studies of gastrointestinal healing. The use of rats from one specific strain diminishes biological variation.

Operative technique

Great care was taken to perform the anastomoses in anatomically well defined sites in the colon since the amount of collagen varies in different parts (Jiborn et al. 1978a; Blomquist et al. 1984b). In the small intestine no variations in collagen content were found within the jejunum or the ileum and the anastomotic sites were allowed to vary within a distance of 3 cm depending on the vascular anatomy in the mesentery. The procedure included ligation of the marginal artery. Care was taken to standardize the number of sutures since this could have some influence on the strength of the anastomoses. Monofilament sutures were used in order to cause a minimum of trauma to the tissue both at insertion and removal.

Mechanical strength

The strength of an anastomosis depends on the sutures, the suture holding capacity of the tissue and the tissue bridging the anastomotic gap. Provided that the suture material used is strong and the knots securely tied the suture factor can be disregarded. During the early phase of healing (0-3 days) the gap is filled by a fibrin clot, the strength of which is negligible. Thus, during this period the strength of an anastomosis with sutures in place is represented by the suture holding capacity of the tissue. The anastomotic strength is then dependent on the number of sutures and the amount and structure of the connective tissue in the intestinal wall encircled by the sutures (Howes et al. 1929; Howes 1940; Tera & Åberg 1976b). Later on accumulation of granulation tissue and collagen adds to the strengthening process. Thus, the strength of the anastomosis with sutures depends on both suture holding capacity of the tissue and

strength of the scar tissue. The strength of the anastomosis after removal of sutures depends only on the scar tissue.

Mechanical properties of an anastomosis can be measured by breaking strength tests on whole specimens or strips (Howes et al. 1929; Hastings et al. 1975a; Le Veen et al. 1976; Deveney & Way 1977; Jiborn et al. 1978c; Gottrup 1980) or by bursting pressure measurements (Chlumsky 1899; Fellows et al. 1951; Herrmann et al. 1964; Nelson & Anders 1966; Letwin et al. 1967; Cronin et al. 1968a; Trueblood et al. 1969; Irvin & Hunt 1974; Bayer & Ellis 1976; Colin et al. 1979; Jiborn et al. 1978b; Brennan et al. 1984; Hesp et al. 1984a).

Bursting strength test was not used in this study because it supplies only limited information about the anastomotic strength. During the first days it is rather a test of the extent of leakage from the anastomosis than a measure of anastomotic strength, and already after one week of healing the burst usually takes place outside the anastomosis (Herrmann et al. 1964; Nelson & Anders 1966; Jiborn et al. 1978b). The tension of the bowel wall depends on the radius of the intestine (Nelson & Anders 1966). Since the anastomotic radius with inverting suture technique is smaller than the radius of the intestine, and the granulation tissue is less distendable than the more elastic intestinal wall, the wall tension will be greater outside the anastomosis (La Place's law). This explains why ruptures occur outside the anastomosis and bursting strength is not a test of anastomotic strength after about one week of healing.

Breaking strength of the whole specimen as advocated by Jiborn et al. (1978c) seems to give accurate information about the mechanical properties of an anastomosis both during the early phase of healing and later on. The importance of standardization of specimen width during measurements in the middle phase of healing has been pointed out by Nilsson (1982) who found proportionality between specimen width and strength during this phase of healing. After ex-

clusion of specimens as reported on page 6, the mean diameter was 6.3 ± 0.6 mm in jejunal, 6.8 ± 0.8 mm in ileal and 7.4 ± 1.2 mm in ileocolic anastomoses. The difference in width between jejunal and ileal anastomoses and between ileal and ileocolic anastomoses was not corrected for when discussing comparisons because the variation of the mean anastomotic width was less than 10%. During the early phase of healing the anastomosis is very fragile, so that strips cannot be used for strength tests. The necessary manipulation would cause damage to the tissue thereby influencing the strength measurements (Gottrup 1980).

Standardization of samples for collagen studies

It would have been interesting to sample only the new tissue bridging the anastomosis, but this is not possible. A relatively large segment of 1 cm was used in order to reduce the influence of methodological error arising from variations in measurement and cutting.

Only in study IV were 0.5 cm segments chosen, because the chief aim of this study was to analyse the extent of changes in mechanical properties.

The results were generally presented as ratios operated/controls. It was therefore essential that the sample excised after healing was as large as the control specimen and that no changes occurred due to contraction or distention. This was controlled by a test where a group of animals had 1 cm segments of the ileum marked already at the operation and excised after one week of healing. These were compared with those from another group of animals where the segments had been measured and excised after one week of healing. Collagen content was found to be equal in comparable segments in these groups indicating that the length was not changed by the healing process. This is in accordance with studies by Glabella et al. (1975; 1977), Stromberg & Klein (1984) and Blomquist et al. (1984b).

The importance of inversion of the edges for the size of the segment can be judged from the results on collagen content in study IV. The middle segment containing the anastomosis had 13% more collagen than the adjoining segments. This segment had two inverted edges. When 1 cm segments were used the division was through the anastomosis and thus only one inverted edge was included. As this was part of a double sized segment only a few percent of the collagen in a 1 cm anastomotic biopsy could have come from inversion. This was neglected.

Collagen studies

Collagen was studied by determination of hydroxyproline. This aminoacid is almost exclusively found in collagen and forms 13.4 weight per cent of the collagen molecule (Neuman & Logan 1950). Only a few other proteins contain hydroxyproline and then in negligible amounts i.e. elastin (Neuman & Logan 1950), the C1_q subcomponent of the complement system (Porter & Reid 1978) and acetylcholinesterase (Rosenberry & Richardson 1977).

Determination of collagen concentration has been widely used in studies on intestinal healing (Cronin et al. 1968a; Wise et al. 1975; Jiborn et al. 1978a; Gottrup 1981b; Hesp et al. 1984a,b). Changes in collagen concentration may, however, be misleading in the interpretation of changes in collagen if used as the only parameter, especially in heterogeneous tissues such as the intestinal wall, since the values will be influenced also by concomitant changes in non-collagenous substances (Irvin & Hunt 1974; Stromberg & Klein 1982a,b; 1984; Blomquist et al. 1984a,b). Determination of collagen content in standardized biopsies gives the real amount of collagen. By relating collagen concentration to changes in collagen content and tissue dry weight it is possible to evaluate changes in non-collagenous substances. In this study hydroxyproline was related to both tissue dry weight and to standardized biopsy, thus giving both collagen concentration and collagen content.

Collagen content is the net result of the original amount of collagen and collagen breakdown and synthesis. Analysis of collagen dynamics thus requires determination of collagen synthesis and/or lysis in addition to collagen content.

Collagen synthesis can be studied either by using radioactively labelled proline as a precursor in the collagen synthesis (Cronin et al. 1968b; Madden & Peacock 1968; Hawley 1969; Madden & Smith 1970; Hastings et al. 1975a; Jiborn et al. 1980a,b; Danielsen & Gottrup 1981; Blomquist et al. 1984b) or by determination of a specific enzyme in the biosynthesis of collagen, protocollagen proline hydroxylase (PPH). Quantitative analysis of this enzyme has proved to be a useful indicator of the rate of collagen synthesis in a variety of tissues (Peterkovsky & Udenfriend 1963; Halme et al. 1969; Uitto et al. 1969; Stein 1969), but has not been generally used in studies on intestinal healing.

Incorporation of proline is one of the first steps in collagen synthesis. After synthesis of the peptide chain a very constant proportion of the proline, 90-100 amino acids/ α -chain, is hydroxylated to hydroxyproline in the presence of PPH (Prockop et al. 1979). Thus hydroxyproline, as such, is never incorporated into collagen (Stetten 1949) but is always derived from hydroxylation of already incorporated proline.

Collagen breakdown can be studied using a prelabelling technique. This requires multiple injections of radioactively labelled proline from early life on. All tissues containing collagen will then be radioactively labelled to some degree. The radioactively labelled hydroxyproline (hypro) can be determined at the beginning of an experiment and after different time intervals. The differences in radioactivity should give a measure of the breakdown of collagen. The technique, however, has important disadvantages in our experimental model. The trauma in connection with intestinal anastomoses will result in a breakdown of collagen from both the abdominal and intestinal wounds. This gives rise to a new pool of

radioactively labelled proline which may be reutilized in collagen synthesis (Klein & Weiss 1966; Klein 1968; 1969; Irvin & Hunt 1974). This causes difficulties in interpretation of results. Furthermore, it is an expensive technique requiring much more isotopes than the pulslabelling technique.

In this study collagen synthesis was investigated by incorporation of radioactively labelled proline after a single dose injection using an incorporation time of 24 hours, which was found to be the optimal time for in vivo incorporation (Jiborn et al. 1980a). The same technique has been used by Cronin et al. (1968b), Hawley (1969), Hastings et al. (1975a), Danielsen & Gottrup (1981) and Blomquist et al. (1984b) in intestinal healing and for studies on skin incisions by Madden & Peacock (1968) and Madden & Smith (1970). Collagen synthesis was expressed as specific activity of collagen (dpm ^3H hypro/ μmol hypro). Collagen synthesis was also calculated as dpm ^3H hypro/standardized biopsy, which gave almost identical results. Relatively lower values of specific activity after one week of healing are explained by the increasing amount of collagen in the intestinal wall during healing.

Protein synthesis was expressed as total radioactivity per biopsy (total dpm ^3H /biopsy) when possible (III). When biopsies were not comparable due to different compositions, as in ileum and colon, (study V) the rate of synthesis was expressed in relation to tissue dry weight.

RESULTS

Postoperative changes of body weight

Animals with jejunal and ileal anastomoses had an equal postoperative weight development. Seven percent of the body weight was lost during the first 24 hours. The preoperative animal weight was restored between days 4 and 7 (II, III, IV). Weight reduction after colonic anastomoses was significantly greater than after ileal anastomoses on the second postoperative day (8 and 3% respectively, $p < 0.01$) and on the fourth postoperative day (4 and 2%, $p < 0.05$). When two anastomoses, one in ileum and one in colon, were made the mean body weight loss on postoperative day 4 was the same as in animals with a colonic anastomosis only (V).

Anastomotic complications

In 182 animals with ileal anastomoses complications occurred in 31 (17%). Two animals died from anastomotic disruption and peritonitis on the fourth postoperative day. The other complications were: dilatation of the bowel (14) suture line dehiscence (4,) abscess (5) and granuloma (6).

In 95 animals with jejunal anastomoses complications occurred in 14 (15%) and in 67 animals with ileocolic anastomoses 15 complications (22%) occurred. The same proportion as in ileal anastomoses of different types of complications was noted.

Intact small intestine

Breaking strength (I)

Breaking strength of intact jejunum was 1.5 ± 0.3 N and that of ileum was 1.9 ± 0.3 N ($p < 0.01$).

Collagen content and concentration (II-VI)

Hydroxyproline content was different in jejunum and ileum, 80 and 100 μg hydroxyproline/cm. No variations within the different intestinal parts were observed.

The above mentioned values of hydroxyproline content correspond to a collagen concentration of 6% in jejunum and 8% in ileum using the conversion factor 7.46 reported by Neuman & Logan (1950). The amount of collagen in intact jejunum and ileum correlated to the strength of the intestinal wall.

Early phase of small intestinal healing (0-3 days)

Anastomotic strength (I, IV)

The strength (suture holding capacity) of the newly performed jejunal or ileal anastomoses was about 65% of that of the intact jejunum or ileum, when anastomoses were made with polypropylene sutures, but 50% when silk was used for suture (IV). Breaking strength in both jejunal and ileal anastomoses successively decreased during the first three postoperative days falling to 15% of that of the newly performed anastomosis on the third day.

Changes in collagen and non-collagenous substances (II, III, V)

After anastomosis tissue dry weight increased in all segments of ileum by 20-40%. Collagen content also increased by 20-40%. Consequently collagen concentration remained unchanged. Collagen synthesis was uniformly increased four to five-fold in all segments. Protein synthesis increased five to seven-fold in all segments. Similar changes in tissue dry weight and collagen content were observed in the jejunum but the increase in collagen content was not significant. Collagen synthesis was increased already on the second postoperative day after ileal anastomoses at distant sites such as the stomach and the colon. (This increase progressed even further at least up to day 7).

Middle phase of small intestinal healing (4-28 days)

Anastomotic strength (I)

a) With sutures

From the third to the fourth postoperative day breaking strength increased equally and significantly in ileal and jejunal anastomoses. A further rapid increase was noted up to the seventh postoperative day, when breaking strength of

the anastomosis reached the strength of the newly performed anastomosis. After 14 days the strength of the jejunal anastomosis with sutures was equal to or stronger than that of the intact jejunum. Ileal anastomoses reached the strength of the intact ileum after four weeks.

b) Without sutures

On day 7 the breaking strength of the tested ileal anastomoses without sutures was about 50% of those with sutures in place. In 14 day-old ileal anastomoses both with and without sutures the breaking strength was equal.

Changes in collagen and non-collagenous substances (II, III, V)

Tissue dry weight increased further in all segments up to day 7 but predominantly in the anastomotic segments. No increase was found later.

Collagen content was rather uniformly increased on day 4 but on day 7 the increase was localized more to the anastomotic segments. Collagen concentration remained unchanged or increased slightly.

Collagen synthesis increased further in the anastomotic segments where a nine-fold increase was noted. In the other segments the rate of synthesis remained at the same level as in the early phase.

Protein synthesis increased three to five-fold fairly uniformly during the first week.

Thus the more localized increase of dry weight from day 4 to day 7 in the anastomotic segments seems to be derived from collagen deposition.

Extent of mechanical changes (IV)

The suture holding capacity approximately 3.5 mm from the anastomoses was unchanged on day 3.

After one week of healing the suture holding capacity of the ileum approximately 3.5 mm from the anastomoses was increased by 40% compared with that of the newly performed anastomosis ($p < 0.01$).

Comparison of changes in collagen and non-collagenous substances in ileal and left colonic anastomoses (V)

Hydroxyproline content in the intact left colon was 150 μg hydroxyproline/cm, but variations were observed between different locations. This corresponds to a collagen concentration of 11% using the factor 7.46 as reported by Neuman & Logan.

Tissue dry weight increased more and earlier around left colonic anastomoses than ileal anastomoses. Collagen content was increased in all colonic segments already on the second postoperative day. In the ileum collagen content was significantly increased only in the two anastomotic segments on the second day. On day 4 collagen content was increased in all segments of both ileal and colonic anastomoses. Collagen concentration was decreased in the two anastomotic segments (3+4) in the left colon after anastomoses on both days 2 and 4. No decrease in collagen concentration was observed in the ileum.

Mechanical and biochemical changes in ileocolic anastomoses (VI)

a) Mechanical changes

The strength of newly performed ileocolic anastomoses was 75% of intact ileum. Breaking strength decreased by 80% up to the third day. Thereafter a very rapid increase was noted, resulting in a higher anastomotic strength than in the newly performed anastomoses ($p < 0.01$) already on day 7 when it was equal to the strength of the intact ileum.

b) Biochemical changes

After anastomosis the tissue dry weight of the ileal wall was increased by 30% on the third day and increased further up to day 7 primarily in the anastomotic segment. Collagen

content remained unchanged up to the third day but increased up to day 7 reaching the same amount and extent as previously observed after ileoileal anastomoses. Collagen concentration in the ileum was decreased by 25-30% on the third day, but increased thereafter to the level of the intact intestine on day seven.

GENERAL DISCUSSION

The mechanical strength of the intestinal wall is mainly provided by the fibrous tissue in the submucosa. Only this layer is strong enough to hold sutures (Halsted 1887). Collagen is the chief constituent of fibrous tissue (Peacock & van Winkle 1976; Lord et al. 1977). Collagen concentrations between 8 and 14 percent of the dry weight have been reported in the colon (Cronin et al. 1968a; Hawley 1969; Irvin & Hunt 1974; Jiborn et al. 1978a). In this study collagen concentration was 6 percent in the jejunum and 8 percent in the ileum. The suture holding capacity of the wall of the respective parts of the small bowel correlated well with the concentrations of collagen (II).

The strength of a newly performed anastomosis depends on the suture holding capacity of the intestinal wall i.e. the amount and structure of collagen included in the sutures and further on the number of sutures and the direction of the pull on them (Howes & Harvey 1929; Howes 1940). Since the number of sutures and the technique for testing were standardized in the present investigation, variations in strength during the early phase depended only on changes in the tissue included in the stitches. Extreme loss of anastomotic strength (i.e. suture holding capacity of the tissue) was found during the first three days (I). On the third day the strength of an anastomosis was only 15 percent of that of the newly performed jejunal and ileal anastomoses. This indicated that the tissue encircled by the sutures had undergone marked changes, i.e. breakdown of collagen and/or structural changes in collagen. The loss of suture holding capacity was confined to the closest vicinity of the anastomosis. No reduction of suture holding capacity on the third day was observed when sutures were placed approximately 3.5 mm from the healing anastomosis (IV). These findings are in agreement with observations by Högström et al. (1985b) in studies of mechanical strength of abdominal fascia and colonic anastomosis. They demonstrated that changes in the suture holding capacity occurred all along the incision line and were not confined to the immediate suture surroundings. The

extreme loss of anastomotic strength during the early phase thus seems to be elicited by local changes in the intestinal wall extending along the entire wound edge and can be due to the surgical trauma.

In most of the present studies collagen in the intestinal wall around the anastomosis was studied in biopsies 1 cm in size (II,III,V). These rather large segments of intestinal wall did not show any decrease of either collagen concentration or collagen content in the early phase of healing. It is obvious however, that loss of collagen very close to the anastomosis may escape detection in such large biopsies. In an attempt to study collagen changes closer to the anastomotic line, (IV), 0.5 cm biopsies were taken with the anastomosis in the centre. This meant that only 2.5 mm of the intestinal wall on each side of the anastomosis was included in the biopsy. Even with this biopsy technique decrease of collagen content was not observed but in fact already on day 3 collagen content was significantly increased. Thus evidence of collagen breakdown with net reduction of collagen content was not found in this study. Very limited breakdown of collagen could, however, remain undetected even with a 2.5 mm margin. Smaller biopsies were not attempted because of difficulties in standardization.

Collagen content is the net result of collagen breakdown and collagen synthesis and there remains a possibility of increased breakdown of collagen being outweighed by the observed early increase of collagen synthesis (III). An exchange of old collagen with new could explain the early decrease of anastomotic strength, since the strength of newly synthesized collagen is inferior to that of mature collagen (Peacock 1984). Structural changes alone e.g. changes in supramolecular organization and bonding of collagen were not studied.

The present study does not clarify the mechanism for the changes resulting in decreased early anastomotic strength. There is, however, in other studies some indirect evidence of local breakdown of collagen. The parallelity between

accumulation of polymorphonuclear leukocytes in the wound (Ross 1968) and changes in anastomotic strength may indicate that these cells are of importance. They are known in some species to contain and release proteinases capable of attacking collagen (Harris & Krane 1974; Weissman et al. 1979; Ohlsson 1980). The local activity of proteinase inhibitors might be insufficient in areas with impaired circulation like the wound edge (Ohlsson & Olsson 1977). Injection of proteinase inhibitors has been demonstrated to have a beneficial effect on wound strength development and maintenance in colon and fascia (Delany & Lalor 1976; Young & Wheeler 1983; Högström et al. 1985a).

Collagen increased early after surgery which is in agreement with observations of early increase in the synthesis of collagen in the colon after anastomosis (Jiborn et al. 1980b) and in skin biopsies after wounding (Cohen et al. 1979). In this phase collagen synthesis was evenly increased in all the small intestinal segments and was increased also at remote intraabdominal sites such as the colon and the stomach. Laparotomy and performance of a small intestinal anastomosis thus induces collagen synthesis throughout the entire gastrointestinal tract. So far it has not been studied if collagen synthesis is also increased in other intra- or extra-abdominal organs. The stimulus for the increase in collagen synthesis in the gastrointestinal tract remains obscure, but may be a response to the surgical trauma to the intestine or to the laparotomy per se. The effects of laparotomy alone on the intestine are not known, but increased collagenolytic activity in the mucosa of almost the entire gastrointestinal tract has clearly been demonstrated after experimental colonic anastomosis (Hawley et al. 1970). The increase of collagen synthesis may thus be a response to increased collagenolytic activity. Another possibility is that the increase in collagenolytic enzyme activity and collagen synthesis are two concomitant responses to a stimulus for the general increase of protein synthesis (Gross 1981).

In the middle phase of healing the anastomotic strength increased rapidly. When the anastomoses were tested with sutures in place the strength at day 7 was equal to that of the newly performed anastomoses (I). When the anastomoses were tested without sutures at that time the strength was only 50 percent of the anastomotic strength with sutures in place. Accumulation of collagen bridging the anastomosis must have occurred, but suture holding capacity had also increased compared with day 3. Thus, at least two factors contributed to the rapid increase of strength from the third day on. Firstly, strength increased due to the increasing amount of tissue bridging the anastomoses and secondly, the suture holding capacity of the tissue increased again. It is not possible from these studies to evaluate the amount of the strength that is contributed by the different factors. However, it might well be that the suture holding capacity of the tissue is the dominating factor of anastomotic strength until the tissue bridging the anastomosis has reached the strength of the sutured tissue. This was achieved after 14 days in ileal anastomoses when anastomotic strength measured with and without sutures was equal (I). The observation indicates that suture support is of no importance for the strength of small intestinal anastomoses two weeks after the operation. Thus synthetic resorbable suture material can be safely used for this type of anastomosis.

Correlation between the suture holding capacity of the tissue in the small intestine and an increase in the amount of collagen in the intestinal wall was clearly demonstrated in the fibroplasia phase. Between days 3 and 7 after anastomosis the suture holding capacity of the tissue about 3.5 mm from the healing anastomosis (IV) increased by 40 percent, corresponding well to the increase of collagen content (35 percent). The increase of collagen in the intestinal wall was not only localized to the immediate anastomotic segment but extended at least 3 cm on both sides of the anastomosis (II). In the present study collagen synthesis was still more increased at distant sites such as colon and stomach during the middle phase. However, it could not be evaluated if the

increased collagen synthesis at these distant sites was accompanied by increased collagen content because the biopsies taken here were not standardized. The rather widespread increase of collagen synthesis and collagen content in the small intestine after anastomosis is in agreement with findings of increase of collagen synthesis and increased bursting strength at remote sites in the colon after anastomosis (Cronin et al. 1968a; Jiborn et al. 1978b). However, not all investigators have found this widespread collagen response. Rather localized healing with increase of collagen synthesis and collagen content not exceeding a distance of 2 cm from small or large intestinal anastomoses have recently been reported in rats (Stromberg & Klein 1982a,b). However, these authors investigated collagen metabolism at one time only, three weeks postoperatively, after repeated labelling by ^3H -proline during the second and third weeks after operation. The difference in techniques and times for sampling may explain the apparently conflicting results.

Anastomoses in the jejunum reached the strength of the intact intestine earlier than anastomoses in the ileum. This can be explained by the fact that jejunum has a lower initial strength than ileum. If the healing proceeds at the same rate in different tissues, it is obvious that anastomoses in tissues with initially lower strength should reach the original tissue strength earlier. It is well known that wounds in tissues with a low collagen content regain the strength of intact tissue earlier than wounds in tissues with a high collagen content. Wounds in muscle, bladder, stomach and colon with a relatively low collagen content regained tissue strength earlier than wounds in skin, fascia and tendon, which have a higher collagen content and a different collagen structure (Douglas 1952; Forrester et al. 1969a,b; Adamsons & Kahan 1970; Hirsch 1974; Hastings et al. 1975a,b).

Separation of granulation from wounded tissue cannot easily be made in incised wounds or anastomoses because not only is the amount of new granulation tissue very small but it also

forms a network with the old connective tissue. A correlation between increased collagen content of granulation tissue and increased strength in the phase of fibroplasia has clearly been demonstrated in studies using experimental models with subcutaneous implantation of sponges (Viljanto 1964; Pallin et al. 1975). Breaking strength and its development should thus give an indirect measure of collagen content and synthesis in the anastomosis. Determination of changes in collagen content and synthesis in the intestinal segments containing the anastomosis probably also reflects the events in the anastomotic gap.

In order to examine if there are differences in healing between colon and small intestine a comparative study of the early course of healing in these two intestinal parts was performed with the same standardized operative technique. Basically the same biochemical changes in the intestinal wall were found in the early healing period. There were, however, differences in time sequence and magnitude of events. In the colon both collagen content and non-collagenous substances increased more and earlier after anastomosis (V). Thus the differences observed did not indicate impaired healing in the colon, instead signs to the contrary were found. Observations of a more rapid gain of strength in left colonic anastomoses compared with the present findings in the small intestine have also recently been made (Blomquist et al. 1984a; Hesp et al. 1984a). The previously mentioned indications of a higher frequency of complications after colonic anastomoses cannot thus be explained by differences in healing.

It is only possible to speculate on the causes of the differences in healing between the left colon and ileum. Differences in bacterial content as well as intraluminal bulk may be of importance. It has been found that diminished intraluminal content reduces the increase of collagen content as well as collagen synthesis in the colonic wall (Blomquist et al. 1984b). Changes in intestinal bulk may induce changes in intestinal wall tension. Mechanical stretching of arterial

smooth muscle cells (Leung et al. 1975; 1977) and skeletal muscle preparations (Palmer et al. 1981; Smith et al. 1983) have been shown to elicit increased collagen and protein synthesis.

When an ileocolic anastomosis is performed the intestinal content in the ileum does not visually change but the barrier between colon and ileum is removed. Normally the total number of bacteria in the distal ileum is about 10^3 to 10^7 per ml and in the colon about 10^{11} per g. This steep change can be seen across the ileocaecal valve (Gorbach 1971). Thus in ileocolic anastomoses the ileum is probably exposed to the same bacterial amount as in the colon. In this model the same early increase of dry weight as in ileo-ileal anastomoses was observed, but the concomitant early increase of collagen content was not found and this resulted in decreased collagen concentration. The decrease of collagen concentration in the ileum extended at least 3 cm from the anastomoses (VI). This has not been noted after ileo-ileal anastomoses. Decrease of collagen concentration occurs, however, regularly after anastomoses in the left colon (Cronin et al. 1968a; Jiborn et al. 1978a; 1980a,b; Blomquist et al. 1984a; V). Thus in an ileocolic anastomosis the anastomosed ileum assumes the healing pattern of the colon. This indicates that the intestinal content and especially its bacterial count influences collagen metabolism in the intestinal wall after an anastomosis has been carried out. Since collagen synthesis was not determined in this investigation it is not possible to conclude whether the lower content of collagen is the result of depression of collagen synthesis or increase of collagen lysis.

The same decrease in breaking strength up to the third day was observed both in ileocolic anastomoses and in ileoileal anastomoses (I, VI). The regain of anastomotic strength was, however, faster in ileocolic than in ileoileal anastomoses (I, VI). Already on day 7 the strength of the anastomosis with sutures in place was higher than the strength of the newly performed anastomosis and equal to that of the intact

ileum. At this time the break took place outside the anastomosis in 40 percent of the tests performed, a phenomenon not observed until day 28 in ileoileal anastomoses. It is not known if this more rapid strength development is caused by changes in the intestinal wall or by the tissue in the anastomotic gap, since the anastomotic strength was measured only with sutures in place. However, as the increase of collagen in the ileal wall was not different from that observed after ileoileal anastomosis, changes in the tissue bridging the anastomosis may have occurred. Evaluation of this would require testing of anastomoses without sutures.

Not only decreased collagen concentration in the ileal wall on the third day but also the increased breaking strength on day 7 after ileocolic anastomoses may be mediated by bacterial content. The role of bacteria as a mediator of changes in collagen metabolism is supported by the fact that intraluminal bacteria have the ability to penetrate into and through the intestinal wall. This has been found in the chemically irritated and paralyzed bowel, where intraluminally administered radiolabelled bacteria could be found after some time in the peritoneal fluid (Schweinburg et al. 1950). Changes in collagen metabolism have been demonstrated after deposition of bacteria intraperitoneally around healing anastomoses of the colon. Reduced amounts of newly synthesized collagen were found on the third day (Irvin 1976). Decreased collagen concentration in intestinal biopsies on the third day of anastomotic healing has been found after infection (Yamakawa et al. 1971; Hawley 1973; Hesp et al. 1984b). It has also been shown that strength development in skin incisions is faster after limited contamination with bacteria (Tenorio et al. 1976; Raju et al. 1977; Gruber et al. 1981). Both bacterial type (Tenorio et al. 1976) and concentration (Raju et al. 1977) seem to be of importance for this reaction. Further it has been shown that bacterial endotoxin can stimulate collagen synthesis in vivo (Freihoffer et al. 1960; Hunt et al. 1984). In vitro it has been demonstrated that endotoxins can stimulate macrophages to release fibroblast activating factors which cause increase of collagen synthesis in fibroblasts (Wahl 1981).

As was earlier pointed out anastomotic dehiscence may be due to decreased ability of the intestinal wall to hold sutures or to impairment of granulation tissue formation in the wound gap. While defects in healing have generally been held responsible several findings in the present thesis indicate that changes in the intestinal wall near the anastomosis are of major importance for anastomotic integrity.

In conclusion:

- Breaking strength of small intestinal anastomoses decreased rapidly during the first three days.
- Decrease of mechanical strength was confined to the closest vicinity of the transection.
- No decrease in collagen content was found during this period.

- Increase of breaking strength was noted from the third day on.
- This increase can be correlated to deposition of collagen in the anastomosis and in the intestinal wall.
- Improved suture holding capacity of the intestinal wall one week after anastomosis correlated to increased collagen content.
- Performance of an anastomosis resulted in an early increase of collagen synthesis not only in the vicinity of the anastomoses but also in distant sites such as stomach and colon.
- Collagen accumulated earlier and to a greater extent after anastomoses in the left colon than in ileum.

- Changes in bacterial count in the intestinal content (ileocolic anastomoses) probably alters collagen metabolism and strength development in the ileal wall.
- Changes in the intestinal wall near an anastomosis are of major importance for anastomotic integrity.

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I

BREAKING STRENGTH OF SMALL INTESTINAL ANASTOMOSES

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SUMMARY

The breaking strength of standardized small bowel anastomoses at different times after surgery was studied in the rat. The anastomotic strength with sutures in place successively decreased during the first 3 postoperative days to approximately 15 percent of the immediate postoperative value. This indicates a rapid decrease in the suture holding capacity of the gut wall in the early postoperative course. From the fourth day onward a rapid increase in strength was recorded. This could be due not only to deposition of collagen in the tissue bridging the anastomosis but also to the regained capacity of the gut wall to withstand tearing forces. After 14 days the strength of the anastomosis was due mainly to healing, and the relative contribution from the sutures was negligible. It may be that tearing of sutures through the tissue is more important than defective healing for anastomotic complications.

INTRODUCTION

The healing process in skin and fascia has been extensively studied (1,2). Findings from such studies have largely been assumed to be valid for the healing process in other, less homogenous tissues such as the gut. However, recent findings (3-9) indicate that gastrointestinal healing differs in several aspects from healing in skin and fascia. The healing process in small intestinal anastomoses has been poorly studied. Clinically, anastomoses of the small intestine are known to have a lower complication rate than anastomoses of the colon. The purpose of this investigation was to determine the breaking strength of anastomoses in the small intestine at various times after surgery and to evaluate the importance of suture support.

MATERIAL AND METHODS

Two hundred twenty-three male Wistar rats weighing 264 ± 32 g were used. In 208 rats 1 cm of small intestine was resected and an end-to-end anastomosis performed. Fifteen unoperated rats served as controls. The rats had no preoperative preparation. Clean but not sterile instruments were used. Anesthesia was induced with intraperitoneally administered chloral hydrate (36 mg/100 g body weight). The abdomen was shaved and entered through a low midline incision 4.5 cm long. After ligation of the appropriate blood vessels but before resection, two presection sutures were placed according to the method of Halsted (10): one on the mesenteric and the other on the antimesenteric border (Fig. 1). These sutures were then used as stay sutures. The bowel was anastomosed with 14 to 16 inverting sutures of 7-0 polypropylene (Surgilene^R). A magnifier was used while performing the anastomosis. The abdominal wall was closed in two layers. Postoperatively the rats were allowed food and water ad libitum.

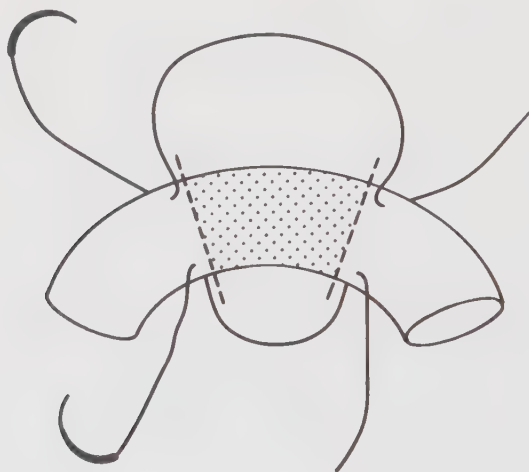


Fig. 1. Presection sutures used as stay sutures.

Group I. Jejunal anastomoses

In 89 animals the anastomoses were made in the upper part of the jejunum, 5 to 8 cm from the pyloric region.

Group II. Ileal anastomoses

In 119 animals the anastomoses were made in the lower part of the ileum at a distance of 5 to 8 cm from the ileocaecal valve. The animals were killed with an overdose of ether, and the intestine was dissected along the mesenteric border and freed of adhesions. The anastomoses were tested in a specially constructed tensiometer which provided a constantly increasing force from 0.03 to 0.05 N/s (in the interval from 0 to 3 N). Anastomoses were tested on the 1st, 2nd, 3rd, 4th, 7th, 14th, and 28th days, as well as immediately after the operation. On the 7th and 14th days one group of animals was also tested after removal of the sutures. The sutures were removed under an operating microscope to minimize trauma to the gut wall. After clamping the intestine in the tensiometer, the diameter of the specimen was determined by a caliper. The mean diameter of the ileal anastomoses was 6.8 ± 0.8 mm and the mean value of the jejunal anastomosis was 6.3 ± 0.6 mm. Dilatation of the intestine was easily detected. The range of values of dilated intestine was 8.7 to 11.4 mm. These animals were excluded.

Statistical methods

In the statistical analysis of the results, the mean values, the standard deviation, and the standard error of the mean were calculated. Comparisons between groups were performed using the Student's t-test for unpaired observations.

TABLE I. Jejunal anastomoses with sutures

Postoperative day	Number of tests	Breaking strength* (N)
Control rats	15	1.49 $\pm$ 0.31
0	8	1.00 $\pm$ 0.25
1	10	0.33 $\pm$ 0.15
2	8	0.20 $\pm$ 0.07
3	11	0.15 $\pm$ 0.02
4	9	0.49 $\pm$ 0.18
7	9	1.17 $\pm$ 0.26
14	9	1.72 $\pm$ 0.32
28	8	1.46 $\pm$ 0.42

* Values indicate the mean $\pm$ standard deviation.

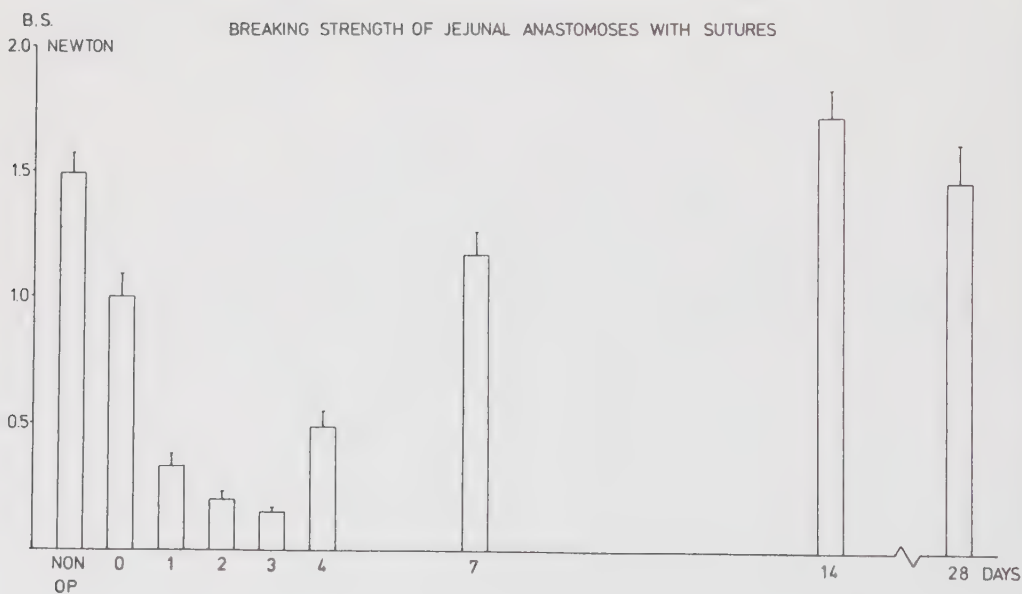


Fig. 2. Breaking strength (B.S.) of jejunal anastomoses in the rat with sutures.

RESULTS

Breaking strength of the small intestine of unoperated control rats

Breaking strength of the jejunum was 1.49 ± 0.31 N and that of the ileum was 1.90 ± 0.27 N ($p < 0.01$).

Breaking strength of jejunal anastomoses

The values of breaking strength with sutures in place at different times after resection and anastomosis of the jejunum are given in Table I and Figure 2. The strength of the newly performed anastomosis was about two thirds of the strength of the unoperated gut wall. During the first 3 days the strength decreased to approximately 15 percent of the immediate postoperative value. From the fourth day on, a rapid increase in strength was recorded. On the seventh day the anastomoses were stronger than immediately after the operation. After 14 days the anastomoses with sutures were stronger than the nonoperated gut. Twenty-five percent of the specimens on that day and on day 28 ruptured outside the anastomosis.

Breaking strength of ileal anastomoses

The values of breaking strength after resection and anastomosis of the ileum with sutures in place are given in Table II and Figure 3. The pattern of strength changes were the same as in the jejunum. The loss of strength during the first 3 days was even greater than in the jejunum. The increase in strength from the third to the fourth day was equal in the jejunum and ileum (0.34 N). On the seventh day the anastomoses with sutures were stronger than immediately after the operation, as was also found in the jejunum. On the 14th day the test values of the anastomoses had not reached those of the nonoperated gut as in the jejunum. All breaks took place at the anastomotic site. After 4 weeks the strength of the ileal anastomoses had reached the level of the nonoperated gut, and the break occurred outside the anastomosis in a third of the tests performed.

TABLE II. Ileal anastomoses with sutures

Postoperative day	Number of tests	Breaking strength* (N)
Control rats	14	1.90 $\pm$ 0.27
0	8	1.20 $\pm$ 0.23
1	9	0.65 $\pm$ 0.22
2	10	0.30 $\pm$ 0.08
3	10	0.13 $\pm$ 0.04
4	8	0.48 $\pm$ 0.13
7	8	1.34 $\pm$ 0.33
14	7	1.42 $\pm$ 0.49
28	9	1.73 $\pm$ 0.64

* Values indicate the mean $\pm$ standard deviation.

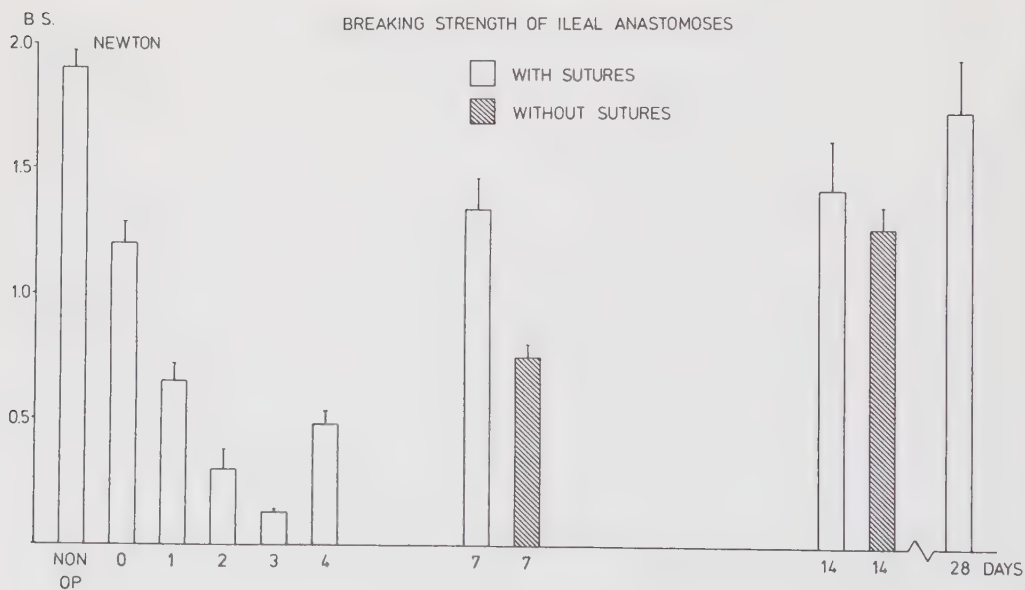


Fig. 3. Breaking strength (B.S.) of ileal anastomoses in the rat. Unshaded columns = with sutures; shaded columns = without sutures.

The breaking strength of ileal anastomoses without sutures was tested on 7 and 14 day old anastomoses on the ileum. The values are given in Table III. The mean value of the breaking strength on the seventh day without sutures was only 56 percent of the mean value of the breaking strength with remaining sutures. In 14 day old anastomoses there was no statistical difference in breaking strength of anastomoses with and without sutures. The mean value of breaking strength without sutures was 1.27 N compared with 1.42 N with sutures.

TABLE III. Ileal anastomoses without sutures

Postoperative day	Number of tests	Breaking strength* (N)
7	9	0.75 $\pm$ 0.16
14	12	1.27 $\pm$ 0.08

* Values indicate the mean $\pm$ standard deviation.

COMMENTS

To evaluate the strength development of intestinal anastomoses, we chose to determine the breaking strength of anastomoses performed in a standardized fashion using a constant number of sutures. Bursting pressure measurements are unsuitable for this purpose, because they only allow evaluation during the very early period after surgery (11,12). In addition, it is not possible to study bowel strips (13), since manipulation of the intestine causes damage to the anastomoses, especially during the early postsurgical period.

Evaluation of breaking strength using the entire segment of the intestine has been recommended by Jiborn et al. (14).

This requires use of a very standardized operative method. Breaking strength during the first 3 days depends on the number of sutures, the direction of the pull on them, and the amount and structure of connective tissue in the intestinal wall (15-17). The number of sutures per anastomosis should if possible be the same. In this study much care was taken to obtain the same number of sutures per anastomosis. All stitches were inserted through the collagen-rich submucosal layer (10).

Studies were performed on anastomoses both in the jejunum and the ileum. Although the strength of the nonoperated intestine was greater in the ileum, the changes in the strength after operation were essentially the same. The newly performed anastomosis had about two thirds the strength of the nonoperated gut wall. Both jejunal and ileal anastomoses rapidly lost strength during the first 3 days after surgery. On the third day the strength of the anastomoses was reduced by about 85 percent of the immediate postoperative value. This loss of strength was due to diminished ability of the sutured intestinal wall to withstand tearing forces. The cause of this impairment has not been clarified to date. However, as the strength of the intestinal wall largely depends on its content and structure of collagen (18), it seems reasonable to assume that alterations of collagen occurred.

From the fourth to the seventh days there was a rapid increase in anastomotic strength. During that time, formation of new collagen in the wound began. The new collagen undoubtedly contributed to the increase in breaking strength but did not explain it in full. Comparing strength of the anastomoses with sutures removed and with sutures in place on the seventh postoperative day showed that about half of the strength was due to healing and the other half to the sutures. This means that between the fourth and the seventh days, in addition to the formation of new collagen in the wound, there was also improved ability of the intestinal wall to withstand tearing forces. After 14 days the contri-

bution by the sutures was minute as no significant difference was found between anastomoses tested with sutures and those without sutures.

The observations made indicate that suture support is of little or no importance to the strength of small intestinal anastomoses 2 weeks after surgery. Thus, synthetic resorbable suture material can be safely used for such anastomoses. Further, the anastomoses are weakest 3 days after surgery. When this observation is related to the experience that anastomotic dehiscence is most commonly diagnosed 4 to 5 days after surgery, it may well be that tearing of sutures through the tissues is more important than a defect in the healing for anastomotic complications.

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II

CHANGES IN COLLAGEN CONTENT OF THE SMALL INTESTINAL WALL FOLLOWING ANASTOMOSIS

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SUMMARY

Collagen content of small intestinal anastomosis in the rat was subjected to investigation in relation to previously reported changes in suture-holding capacity. The decrease in suture holding capacity during the first three days previously observed (inflammatory phase) did not correlate to collagen content during this period.

The increase in suture-holding capacity from the third to the seventh day (the phase of fibroplasia) was however well correlated to an increased collagen content in the bowel wall in both ileum and jejunum. Anastomotic strength and collagen content correlated well from the third day on. Deposition of collagen not only in the wound but also in the intestinal wall and within the loop of the suture during the phase of fibroplasia is the likely explanation for the rapid increase of suture-holding capacity during this period.

INTRODUCTION

The mechanical strength of an anastomosis is during the first days mainly due to the suture holding capacity of the gut wall (1,2). In a previous experimental study we recorded a decrease of about 85% in the suture holding capacity of the small intestinal wall during the first three postoperative days. Thereafter a rapid regain of strength occurred (2). Since the mechanical properties of the intestine are dependent on the amount and structure of collagen in the gut wall (3), it can be assumed that changes in collagen take place around the anastomosis.

This study was undertaken in order to investigate changes in collagen content of the intestinal wall around a small intestinal anastomosis during the healing process.

MATERIALS AND METHODS

Two hundred and twenty-two male Wistar rats weighing 269 ± 46 g were used. Twenty-two rats served as controls. In 200 animals one centimeter of the small intestine was resected and an end-to-end anastomosis performed. The anastomoses were made in one layer using inverting, interrupted 7-0 polypropylene (Surgilene^R) sutures as previously described (2). In 95 rats the anastomosis was performed 5 to 8 cm from the pyloric region (Fig. 1a), and in 105 rats an ileal anastomosis was made 5 to 8 cm from the ileocecal valve (Fig. 1b). The animals were weighed at the beginning and at the end of the experimental period.

Groups of animals were killed by an overdose of ether at one, two, three, four, seven, 14 and 28 days postoperatively. Anastomotic dehiscence, abscess or granuloma were registered and the width of the anastomosis was measured in a standardized manner with a caliper. Animals with complications or bowel dilatation according to previously defined criteria (2) were excluded from further study.

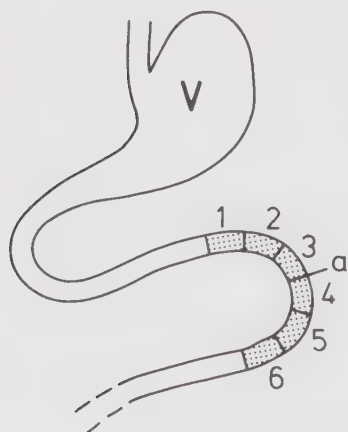


Fig. 1a. Location of jejunal anastomosis (a). Number 1, 2 and 3 = preanastomotic biopsies; 4, 5 and 6 = postanastomotic biopsies. V = stomach.

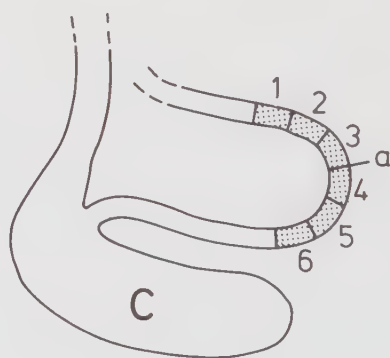


Fig. 1b. Location of ileal anastomosis (a). Number 1, 2 and 3 = preanastomotic biopsies; 4, 5 and 6 = postanastomotic biopsies. C = cecum.

The intestine was dissected along the mesenteric border and freed of adhesions. Three consecutive, one-centimeter segments were cut out on both sides of the anastomosis (Fig. 1a, b). The sutures were removed and the mucosa was scraped with a pair of scissors to remove mucus and intestinal content. Dry weight of the specimens was recorded and hydroxyproline was determined according to Stegemann and Stalder (4) after hydrolysis in 6 N HCl for 6 hours. Hydroxyproline content was calculated as μg hydroxyproline/standardized biopsy.

Statistical methods

The mean and standard deviation were calculated. Student's t-test for unpaired observations was used for analysis of the results. $P < 0.05$ was considered significant.

RESULTS

Postoperative weight development was equal in both groups. Seven percent of the body weight was lost during the first 24 hours, after which there was an equivalent regain. The preoperative weight of the animals was restored between day four and seven.

Anastomotic complications occurred in 14 of 95 animals with jejunal anastomosis (15%) and in 23 of 105 with ileal anastomosis (22%). Five of these 200 animals died from anastomotic dehiscence between the second and fourth postoperative day (2.5%). The remaining animals were analyzed in subgroups consisting of 8 to 16 animals.

Hydroxyproline content Results from jejunal anastomoses are presented in figure 2 as the sum of hydroxyproline content in the two anastomotic segments (3+4) as ratios between operated and controls. During the first three postoperative days there were only small changes in hydroxyproline content. From day four on significant increase of collagen content in the anastomotic segments occurred. On day seven the collagen content was twice that of controls.

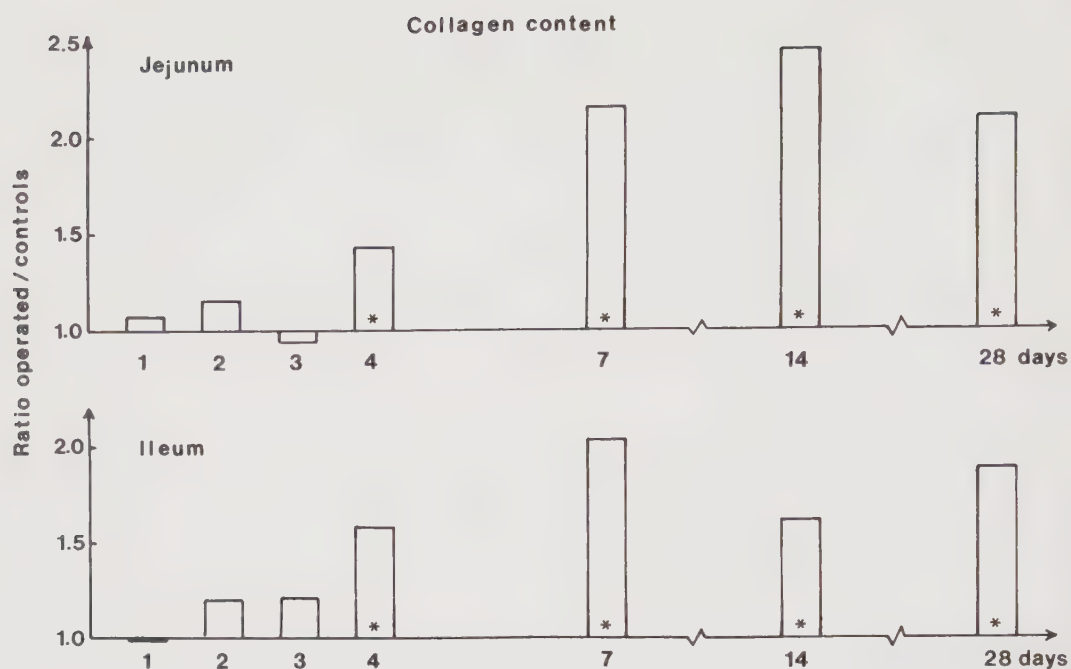


Fig. 2. Hydroxyproline content in the jejunal and ileal wall. Sum of the two anastomotic segments (3+4), recorded as ratio of means of operated to control animals. * denotes significant difference.

Results from ileal anastomoses are presented in figure 2. A similar development of collagen content was found in the ileum as in the jejunum. The increase was first localized to the anastomotic segments, but on day 28 a significant increase was found in all the six segments compared to controls (Fig. 3). The extent and development of collagen accumulation on four representative days is shown in figure 3.

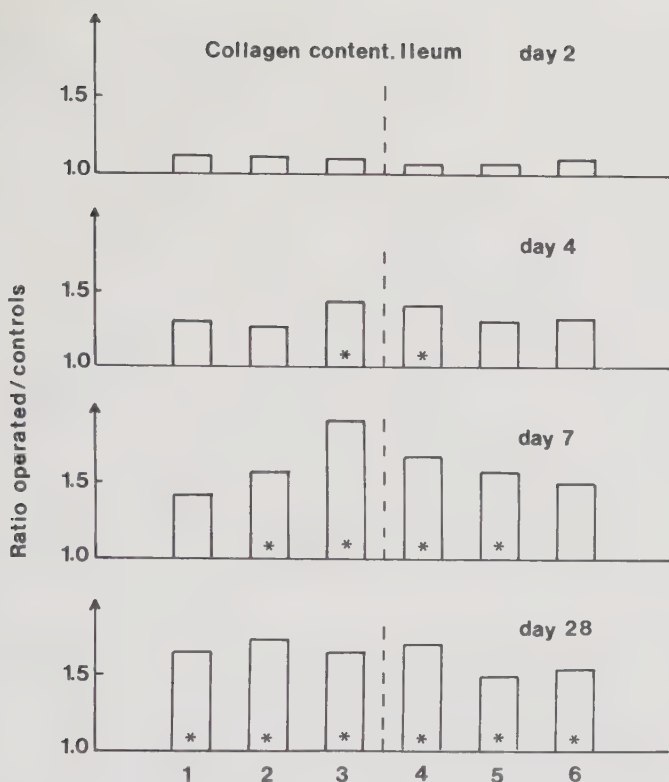


Fig. 3. Collagen content of ileal biopsies expressed as a ratio between operated and control animals. 1-3 = preanastomotic biopsies, 4-6 = postanastomotic biopsies. Interrupted line indicates anastomotic site. Significant differences compared to controls marked with an *.

DISCUSSION

In a previous study we recorded a decrease of about 85% of the suture-holding capacity on the third postoperative day in small intestinal anastomoses (2).

No significant changes in collagen content were found in either jejunal or ileal wall during the first three days postoperatively in the present study. Thus the extreme loss of suture holding capacity did not correlate to collagen content in one cm biopsies. It cannot be excluded that

changes in collagen content might occur in the closest vicinity of the anastomosis without being detected with this biopsy technique.

Loss of suture holding capacity might be due to a very local effect of sutures cutting through the gut wall (5). This effect could be mediated by the inflammatory cells around the suture. Phagocytes (leukocytes and monocytes) are known to leak or secrete enzymes, especially neutral proteases, when exposed to a phagocytic stimulus. These proteases activate collagenolytic enzymes which may start a breakdown of collagen in the connective tissue before they are inhibited by local antiproteases (6). This local process may take place around the suture as an inflammatory response to the trauma of the stitch and transection of the bowel.

Our findings of unchanged collagen content around the anastomoses during the early phase of small intestinal healing are in contrast to observations from healing of the colon. In the colon collagen concentration has been reported to decrease about 40% after operation (7,8). When collagen content in standardized biopsies was studied (9) a reduction of approximately 25% was found in the anastomotic region. Wise et al. (10) studying anastomotic healing in both small and large intestine in dogs noticed the same difference measured by collagen concentration between different parts of the bowel. They concluded "a better collagen response" in the small than in the large intestine. Gottrup (11) in his study of the stomach and duodenum found different collagen concentration in the rumen than in corpus and duodenum. The different parts also showed different response after wounding as measured by collagen concentration. The differences in collagen response could be due to a higher collagenase activity in the colonic wall than in the small intestine and the stomach as reported by Hawley (12).

In a previous report we found a very rapid regain of the suture holding capacity from 15 to roughly 50% of the immediate postoperative value between day three and seven (2).

This increase of the suture holding capacity correlates with the deposition of collagen in the intestinal wall remote from the anastomotic site (Fig. 3). This increase of collagen content in the intestinal wall and thus within the loop of the suture is the likely explanation for the increasing suture holding capacity observed from the third day on (2). The anastomoses thus gained strength partly due to anastomotic healing, partly due to regained capacity of the bowel wall to withstand tearing forces.

The findings of collagen deposition outside the immediate anastomotic site are consistent with those of Cronin et al. (7). They reported an increased collagen concentration in the intestine after left colon anastomosis, resulting in an increased bursting strength of the uninjured large bowel.

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III

COLLAGEN METABOLISM IN SMALL INTESTINAL ANASTOMOSIS

Kent Jönsson, Hasse Jiborn and Bengt Zederfeldt

SUMMARY

Collagen metabolism in the small intestinal wall during the first week of anastomotic healing was studied. During this period marked changes in anastomotic strength have been observed. A general and marked increase of collagen synthesis was found throughout the intestine already on the second day. This resulted in increased collagen content. After the fourth day collagen content increased predominantly in the anastomotic region. The extreme decrease of anastomotic strength observed in the early phase of small intestinal healing in previous studies could not be explained. A very local process for the decrease of suture holding capacity is proposed.

INTRODUCTION

The amount and structure of collagen in the intestinal wall is considered to determine bowel wall strength and suture holding capacity (11). In previous experimental studies of small intestinal healing we found a marked reduction of the suture holding capacity during the first three days after surgery but no major changes in collagen content of the intestinal wall (7,8). There was thus no direct correlation between collagen content and suture holding capacity during the first three postoperative days. In an attempt to further elucidate the importance of collagen we have studied collagen metabolism during the first week of small intestinal healing, when marked changes of anastomotic strength have been observed (7).

MATERIAL AND METHODS

Thirty-nine Wistar male rats weighing 226 ± 26 g were used. Six rats were not operated and served as controls. In 33 animals a standardized ileal anastomosis was performed 5 to 8 cm from the ileocecal valve (Fig. 1). The anastomoses were made in one layer using interrupted, inverting 7-0 polypropylene (Surgilene^R) sutures as previously described (7). Body weight was recorded at the beginning and at the end of

the test period. 3.7 MBq (100 μ Ci) L-proline (2-3-4-5³H) (New England Nuclear, specific activity 3.7-4.4 $\times 10^6$ MBq/mmol) were given intravenously 24 hours prior to sacrifice (4). Groups of animals were sacrificed on day two, four and seven postoperatively.

Dilatation of the bowel, abscess and granuloma formation were registered and animals with these complications were excluded from further study (7). The ileum was dissected along the mesenteric border and freed from adhesions. Three consecutive one centimeter segments were cut out on both sides of the anastomosis and the sutures removed (Fig. 1). Three distant biopsies were also taken; from the stomach (glandular part), the jejunum (approximately 8 cm from pylorus) and the colon (at the major flexure) (9). The mucosa was scraped with a pair of scissors to remove intestinal content and mucus. The specimens were dried to a constant weight in an oven at 100° C and dry weight recorded. The samples were then hydrolyzed for 6 hours at 130° C in 6 N

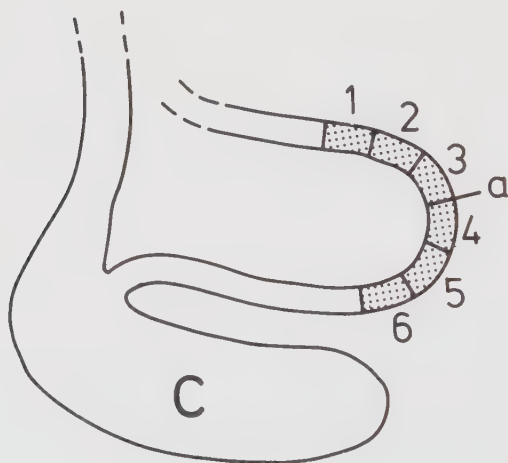


Fig. 1. Location of ileal anastomosis (a). Number 1, 2 and 3 = preanastomotic biopsies; 4, 5 and 6 = postanastomotic biopsies. C = cecum.

HCl and hydroxyproline (hypro) was determined according to Stegemann and Stalder (13). Total radioactivity and radioactivity of ^3H -hypro were determined according to Juva and Prockop (6) as applied to intestinal healing by Jiborn et al. (4).

The following calculations and terms are used in the text and figures.

- 1) Collagen concentration (μg hypro/mg tissue dry weight).
- 2) Collagen content (μg hypro/biopsy).
- 3) Total radioactivity (total dpm ^3H /biopsy) reflecting the synthesis of all proteins including collagen.
- 4) Specific activity of collagen (dpm ^3H hypro/ μmol hypro) as a measure of the newly synthesized collagen in relation to the total amount of collagen in each specimen.

Statistical methods

Mean and standard deviation were calculated. Student's t-test for unpaired observations was used for all comparisons between operated and control groups of animals. $p < 0.05$ was considered significant.

RESULTS

The mean body weight loss was 5% from the operation to the second postoperative day. The operative weight was restored on postoperative day four. On day seven an increase by 6% was recorded.

Four animals with anastomotic complications (12%) were excluded.

Specimen dry weight was significantly increased during the whole period of observation (Fig. 2-4). On day two the increase was rather uniformly distributed among the studied segments. From day four on there was a further increase of dry weight in the anastomotic segments.

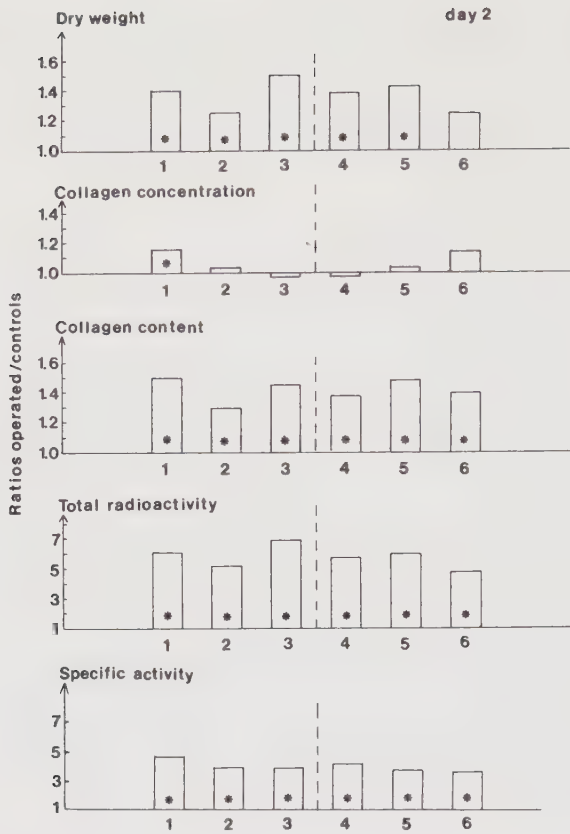


Fig. 2. Dry weight, total radioactivity, collagen concentration, collagen content and specific activity on day 2 after anastomosis given as ratio: operated/controls.

Protein synthesis was markedly and uniformly increased in all the segments with the highest values on day two (Fig. 2-4).

A significant increase of collagen content was seen on the second day in all segments. On the seventh day accumulation of collagen became more pronounced in the anastomotic segments (Fig. 2-4).

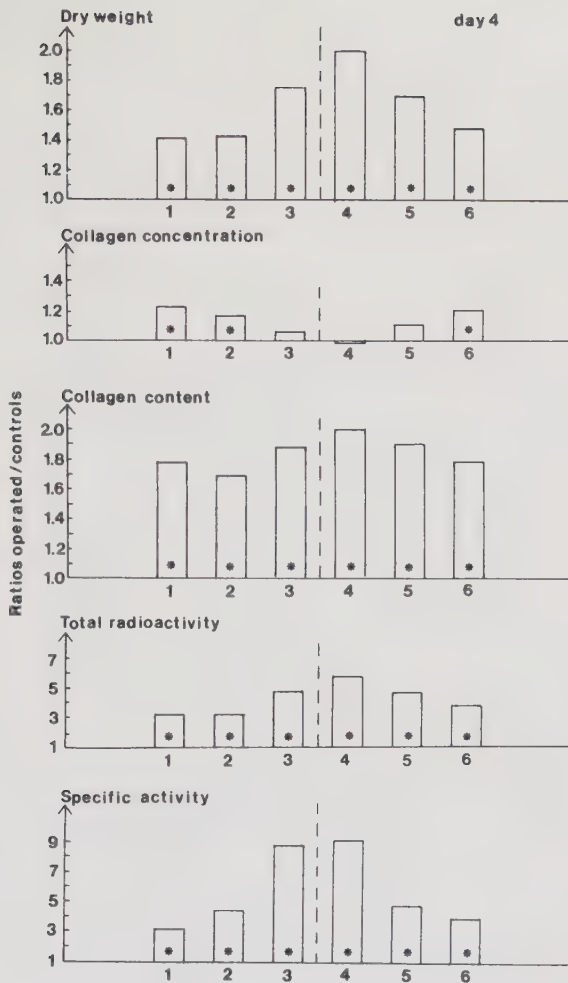


Fig. 3. Dry weight, total radioactivity, collagen concentration, collagen content and specific activity on day 4 after anastomosis given as ratio: operated/controls.

Collagen concentration remained virtually unchanged with a tendency to increase in the segments remote from the anastomoses (Fig. 2-4).

Specific activity of collagen was uniformly increased in all segments two days postoperatively. On day four and seven the increase of collagen synthesis was most marked in the anastomotic segments (Fig. 2-4).

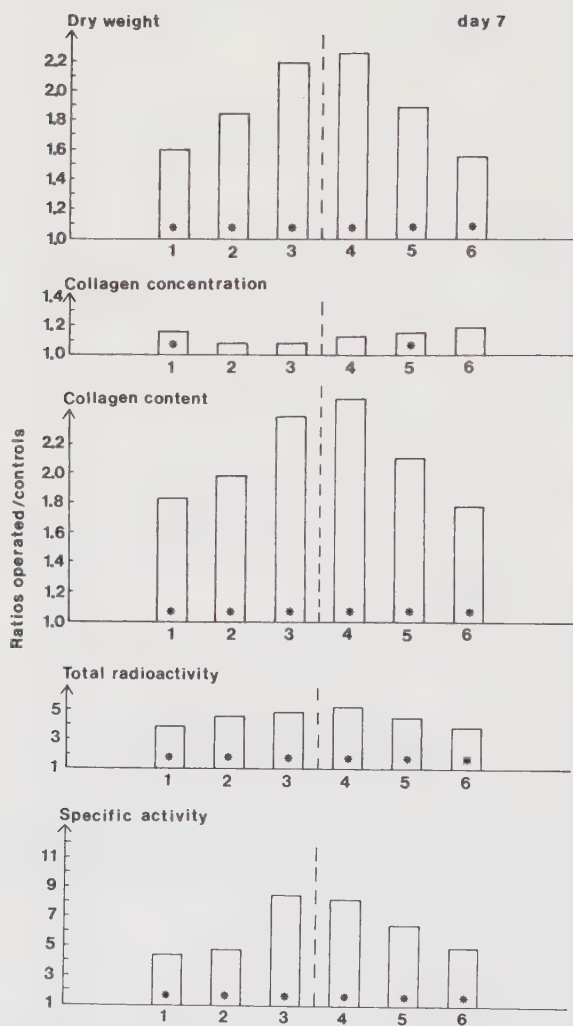


Fig. 4. Dry weight, total radioactivity, collagen concentration, collagen content and specific activity on day 7 after anastomosis given as ratio: operated/controls.

Specific activity of collagen was significantly increased in the biopsies from the stomach, the jejunum and the colon from the second day on (Fig. 5).

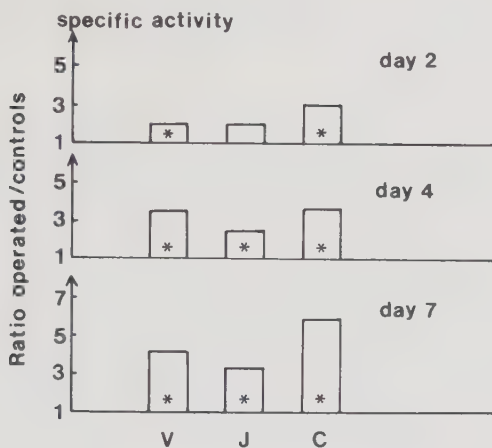


Fig. 5. Specific activity of collagen in the stomach = V, jejunum = J and colon = C on day 2, 4 and 7 given as ratio: operated/controls.

DISCUSSION

In this study collagen synthesis was studied by in vivo incorporation of a collagen precursor. Measurements were made on three different days during the first week of healing and related to collagen content.

Collagen content in each specimen was increased already on the second day. This accumulation of collagen was parallel to a uniformly distributed increase of collagen synthesis during the first days after wounding. As there was an increase of collagen content throughout the investigated period collagen synthesis must have dominated over collagen breakdown. Not only the anastomotic region but also other parts of the small intestine, the stomach and the colon reacted with increased collagen synthesis (Fig. 5). A pro-

gressive increase of collagen content was found in the ileum, but on day seven the collagen accumulation became more localized to the anastomotic segments. The early onset of collagen synthesis without specific localization might be due to a general reaction to the surgical trauma, while the more marked increase of collagen synthesis in the anastomotic segments later on rather represents anastomotic healing.

Our findings indicate that collagen synthesis starts very early after wounding. This is in accordance with recent studies in skin biopsies (2) and intestinal biopsies (5).

Collagen concentration did not increase in the early phase despite increase of collagen content. This is explained by concomitant increase of other substances in this heterogeneous tissue (15), as confirmed by the increase in dry weight of the biopsies. There was an early, marked and general increase of protein synthesis in all the investigated segments of the bowel. The highest values were observed in the first days and thereafter a gradual decrease was noticed. Thus the time course and localization of changes in collagen synthesis and protein synthesis were different. The changes in protein synthesis might be a general response to surgical trauma as is suggested from the early increase in collagen synthesis.

The rapid loss of suture holding capacity in the early course of healing in the small intestine previously reported (7) was not accompanied by any reduction of collagen content but in fact an increase was found. The discrepancy between collagen content and changes in mechanical properties in the early phase could be due to immaturity of the newly synthesized collagen. The rapid increase of strength after the third day would then be mediated not only by increased amount of collagen but also increased polymerisation or maturation of collagen by formation of intra- and intermolecular crosslinks (12). Loss of suture holding capacity could also be due to a very local process initiating collagen breakdown in the closest vicinity of the sutures. With the

technique used in these experiments studying 1 cm biopsies a localized collagen breakdown could be missed, overshadowed as it might be by increased synthesis in the other parts of the biopsies. Such local breakdown of collagen might be mediated by phagocytes leaking or secreting proteases activating specific collagenase (14). These enzymes are allowed only to act very locally before they are inactivated by α_2 -macroglobulin and β_1 -anticollagenase (10,16). Cutting of sutures through tissue would then be a consequence of collagenase acting locally on the collagen fibrils closest to the suture where availability of the inhibitor might be decreased due to impaired circulation. If this is the case cutting of sutures could possibly be prevented by protease inhibitors. In experimental studies administration of protease inhibitors to animals after wounding increased strength of laparotomy wounds (1) and intestinal wounds (3,17).

CONCLUSIONS

- 1) Collagen synthesis and collagen content in the small intestinal wall increased from the second day of operation through day seven after intestinal anastomosis.
- 2) Collagen concentration remained relatively unchanged despite increase in collagen content, as a result of concomitant increase of non-collagenous substances.
- 3) The loss of suture holding capacity could not be explained by changes in collagen content or metabolism. A very local process for cutting of sutures is proposed.
- 4) A marked increase of collagen synthesis was recorded in the stomach and the colon as a distant response probably elicited by the surgical trauma.

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IV

MECHANICAL AND BIOCHEMICAL ALTERATIONS IN THE INTESTINAL WALL ADJACENT TO AN ANASTOMOSIS

Kent Jönsson, Hasse Jiborn and Bengt Zederfeldt



SUMMARY

Mechanical strength of the small intestinal wall approximately 3.5 mm from an anastomosis was studied during the first week of healing and correlated to alterations of collagen content. The mechanical properties of the small intestinal wall approximately 3.5 mm from the primary anastomosis remained constant during the first three days of healing. Previously observed decrease of suture holding capacity of the small intestinal wall thus is a very local process extending less than 3.5 mm from the transection line. After one week of healing the increase of suture holding capacity correlated well with the increase of collagen content in the bowel wall.

INTRODUCTION

Intestinal anastomoses lose a great deal of their initial strength during the first postoperative days (1,2,3,4,5). In the colon this has been related to a loss of collagen around the anastomosis (2,3). In the small intestine, however, the marked reduction of anastomotic strength is not accompanied by measurable loss of collagen (3,6,7). This is surprising as it is generally assumed that the strength of the bowel wall is dependent on the collagen content. One possible explanation is that collagen changes were so limited that they escaped detection in the segments of the bowel wall studied biochemically.

The aim of the present study was to examine the extent of changes in mechanical strength in the intestinal wall early after an anastomosis and correlate this to alterations of collagen content.

MATERIAL AND METHODS

In thirty-four Wistar male rats weighing 237 ± 18 g an ileal anastomosis was performed 5-8 cm from the ileocecal valve, using 14-16 inverting sutures of 7-0 silk. The operative technique was the same as previously described (4), which means placing stitches approximately one mm from the transection line through all layers but the mucosa. Ten animals were sacrificed immediately after anastomosis (day zero) and used as controls. Breaking strength of these anastomoses, i.e. suture holding capacity, was determined in a specially constructed tensiometer (4). In twelve animals the anastomosis was allowed to heal for three days and in another twelve animals for seven days. The animals were then reoperated. Three 5 mm segments were marked with sutures (Fig. 1).

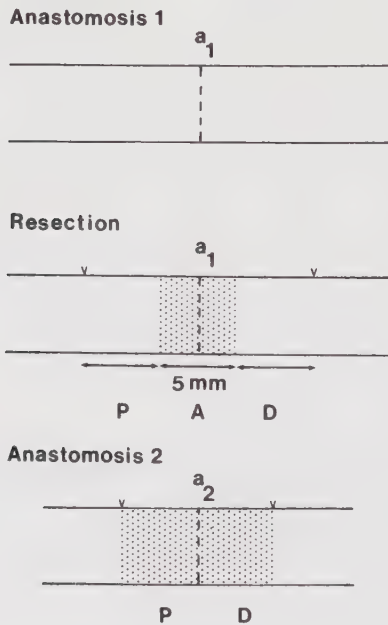


Fig. 1. The ileum was transected and anastomosed. After three or seven days the primary anastomosis including 2.5 mm on each side was resected, and a new anastomosis was performed. Suture holding capacity was then tested immediately, and standardized segments were taken for analysis of collagen content. (A = anastomosis, P = proximal segment and D = distal segment).

The middle segment containing the anastomosis in the centre was freed from adhesions and resected. The intestinal ends were anastomosed using the technique described above and breaking strength of the second anastomosis was immediately determined. The marked segments were excised.

All three segments were dried in an oven to constant weight and dry weights were recorded. After hydrolysis hydroxyproline was determined according to Stegemann and Stalder (8). Collagen was expressed as μg hydroxyproline per standardized biopsy and collagen concentration as g hydroxyproline per mg dry weight. Body weight was recorded at the beginning and at the end of the experimental period.

Statistical methods

The results were analysed by calculating the mean values, the standard deviations and the standard error of the means. Student's t-test for unpaired observations was used for comparisons between groups. $P < 0.05$ was considered significant.

RESULTS

The mean body weight decreased by 3% from the operation to the third postoperative day. On day seven an increase by 7% was recorded.

Anastomotic complications occurred in three animals (13%) which were excluded from further study.

Suture holding capacity (S.H.C.) of the small intestinal wall on day zero (=controls) and in the intestinal wall at a distance of approximately 3.5 mm from the primary anastomosis, three and seven days after operation is given in fig. 2. The strength of the tissue approximately 3.5 mm from the anastomotic line three days after the primary anastomosis was not different from the strength of the intestinal wall on day zero. On the seventh day the mechanical strength at the corresponding location was increased by approximately 40% ($p < 0.01$).

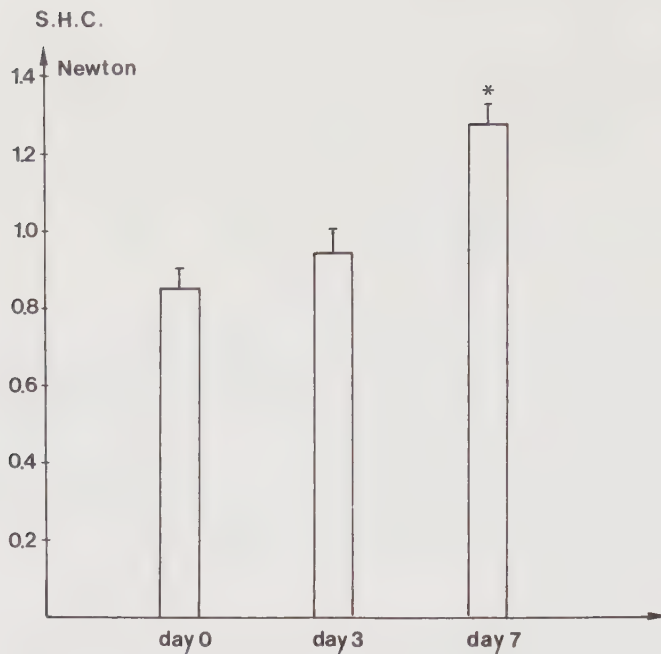


Fig. 2. Suture holding capacity (SHC) of the primary anastomosis of the ileum in Newton ($M \pm SEM$) on day zero and suture holding capacity of the second anastomosis after three and seven days. * denotes significant difference ($p < 0.05$) from day zero.

Tissue dry weight (fig. 3) was significantly increased in all three segments on day three and seven. In the anastomotic segments the dry weight was almost doubled already on day three.

Collagen content (fig. 3) was significantly increased already on the third day after the operation in segment P and A. At day seven the amount of collagen was doubled in segment A and increased by 35% in segments P and D compared with day zero.

Collagen concentration (fig. 3) in the anastomotic segment was decreased on day three compared to controls. On day seven the collagen concentration was equal to that on day zero. In segments P and D there were no changes in collagen concentration.

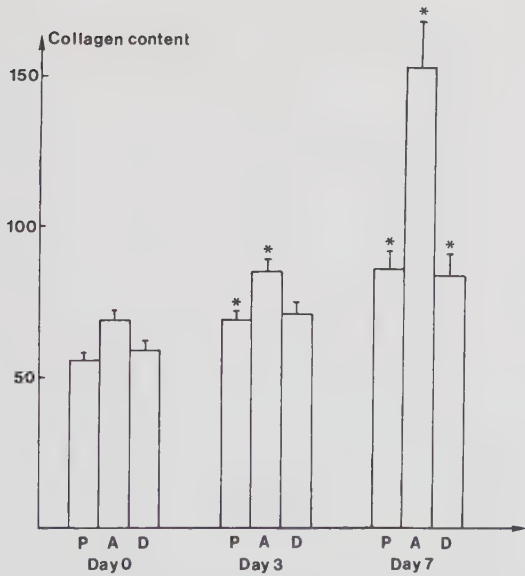
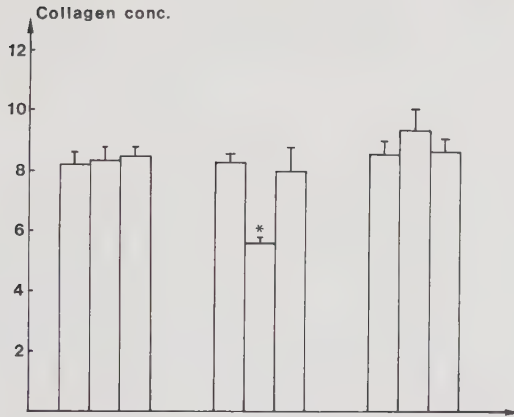
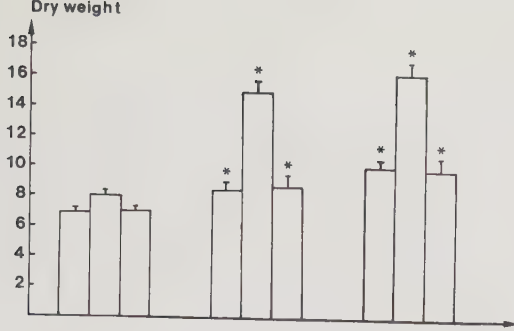


Fig. 3. Tissue dry weight in mg, collagen concentration in µg hydroxyproline per mg dry weight and collagen content in µg hydroxyproline per standardized biopsy (M±SEM) are given in absolute values on days zero, three and seven after anastomosis. (A = anastomotic, P = proximal and D = distal segment). * denotes significant difference from day zero.

DISCUSSION

In a previous study the breaking strength of an ileum anastomosis was found to decrease by 85% during the first three postoperative days (4). The present study shows that at the corresponding time the strength of the bowel wall approximately 3.5 mm from the anastomosis is not decreased. Thus, the previously observed postoperative loss of strength must be due to a process confined to the closest vicinity of the transection line. These findings are in accordance with observations by Högstrom et al. (9) from studies of colonic anastomoses.

The changes of the intestinal wall may occur only around the sutures. However, Högstrom et al. (9) have shown that in abdominal fascia the changes of mechanical strength goes all along the incision line which seems to indicate that it is not the presence of sutures but rather the incision that decides the decrease of mechanical strength.

Incisional wounds are surrounded by a biochemically active zone with a reduction of collagen concentration on both sides of the incision. Adamsons et al. (10) and Gottrup (11) have found this zone to measure five to seven millimeters on each side of the wound and to last for up to three weeks in guinea-pig fascial and rat gastric incisions respectively. In the present study decrease in collagen concentration is noted only in the nearest millimeters from the transection line and only during the first three days. There may be differences between different species explaining the discrepancy in observations. However, it should be noted that in the segment with decreased collagen concentration the collagen content was in fact increased. These findings are explained by a concomitant larger increase of substances other than collagen. It is thus obvious that in the anastomotic segment we do not find any decrease of collagen content, corresponding to the decrease of suture holding capacity during the first days after operation. The apparent discrepancy between strength and collagen content could be due to immaturity of the newly formed collagen.

Deposition of collagen starts very early after wounding (7,12) and contributes already in the first days significantly to the amount of collagen found in the intestinal wall. However, it is obvious that this new collagen does not contribute to the mechanical properties of the intestinal wall on the third day. It is probable that the local breakdown of old collagen is the explanation for the impaired mechanical properties during the first days after anastomosis, but it cannot be excluded that the collagen structure is changed by accumulation in the area of substances other than collagen as is evident from the decrease of collagen concentration despite the increased collagen content.

In previous studies it has been shown that there is an increased collagen synthesis in the intestinal wall after performance of an anastomosis. This increase in synthesis extends several centimeters (6,7,12) from the anastomosis and can be detected also at remote sites (7). An increase in collagen content in all segments studied here is obvious on day seven. This increase in collagen content is paralleled by an increase in mechanical strength after one week at a distance of approximately 3.5 mm from the primary anastomosis. Previously it has been shown that mechanical strength is increased at distant sites from the surgical trauma in the gastrointestinal tract. Thus, in the stomach Harvey and Howes found increased bursting pressure ten days after gastric incisions (13). In later studies increased bursting pressure of the uninjured colon has been ascribed to the newly formed collagen (2,14).

In conclusion, the mechanical strength of the small intestinal wall approximately 3.5 mm from an anastomosis remained constant during the first three days of healing. Previously observed decrease of suture holding capacity of the small intestinal wall, thus is a very local process extending less than 3.5 mm from the transection line. After one week of healing the increase of suture holding capacity (approximately 40% at a distance of approximately 3.5 mm from the primary transection line) correlated well with the increase of collagen content in the bowel wall at that time.

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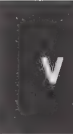
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V

COMPARISON OF HEALING IN THE LEFT COLON AND ILEUM

Changes in collagen content and collagen synthesis in the
intestinal wall after ileal and colonic anastomoses in the rat.

Kent Jönsson, Hasse Jiborn and Bengt Zederfeldt



ABSTRACT

Collagen metabolism was compared after anastomosis in the small and large intestine of rats. Animals were sacrificed 2 and 4 days postoperatively, when three standardized biopsies were taken on either side of the anastomosis. In one group of rats, anastomosis was performed in both ileum and colon and ^3H L-proline was given 24 hours before sacrifice, for studies of collagen synthesis. Animal weight, specimen dry weight, collagen concentration, collagen content per standardized biopsy, specific activity of collagen and total radioactivity were measured. Weight loss was greater after colon than after ileum anastomoses, indicating that the rats were more severely affected by colon surgery than by comparable operations on the ileum. Collagen content increased earlier and more in large intestinal than in small intestinal wall. The earlier activation of collagen synthesis in colon may have been related to differences in intestinal content (e.g. bulk or bacteria).

INTRODUCTION

Anastomoses in the colon are generally considered to cause more clinical problems than do small-bowel anastomoses, which may be the reason why anastomotic healing has been studied more often in colon than in small intestine. In the colon, collagen turnover in the anastomosis and intestinal wall has been investigated in several experimental studies (1-3,5,7-9). Also in this respect the small intestine is much less studied, and the few comparisons between small and large bowel (6,15) have given conflicting results.

The present study was undertaken to compare collagen metabolism in and around the anastomosis in the small and the large intestine during the early phase of healing.

MATERIAL AND METHODS

The experiments were performed on 59 male Wistar rats weighing 272 ± 30 g. The animals had free access to standard laboratory food and water before and after operation. They were anesthetized with chloral hydrate intraperitoneally, 36 mg/100 g body weight. Nine of the rats were sacrificed immediately after operation, and served as controls.

Laparotomy was performed through a midline incision. In 20 rats a 1-cm segment of ileum was resected 5-8 cm above the ileocecal valve and anastomosis was performed with 14-16 inverting sutures of 7-0 polypropylene. These rats were sacrificed on day 2 (n=10) or day 4 (n=10) and used for hydroxyproline analyses. In 21 animals a 1-cm segment of the left colon was resected 3 cm above the peritoneal reflection and anastomosis was performed with the same technique. The rats were sacrificed on day 2 (n=11) or day 4 (n=10) and also used for hydroxyproline analyses. In the remaining nine rats, two anastomoses were performed, one in the ileum and one in the colon at the mentioned sites. These animals were used for study of collagen and protein synthesis. They were intravenously injected with 7.4 MBq (200 μ Ci) L-proline ($2\text{-}3\text{-}4\text{-}5^3\text{H}$, New England Nuclear, specific activity 3.7-4.4 $\times 10^6$ MBq/mmol) 24 hours prior to sacrifice on day 4.

Body weight was recorded at the beginning and at the end of the test period. Previously reported (11) criteria for exclusion (abscess, dehiscence, granuloma and dilatation) were used. The intestine and anastomosis were dissected along the mesenteric border and freed from adhesions. The anastomoses were transected in the middle and three consecutive 1-cm segments were cut out on both sides of the join (Fig. 1). The mucosa was scraped with scissors to remove intestinal content and mucus. The specimens were dried to constant weight in an oven at 100° C and dry weight was determined. They were then hydrolyzed for 6 hours at 130° C in 6 N HCl and hydroxyproline (hypro) was determined according to Stegemann and Stalder (13). Total radioactivity and specific activity of collagen were assayed with the method of Juva and Prockop (10) as applied to intestinal healing by Jiborn et al. (8).

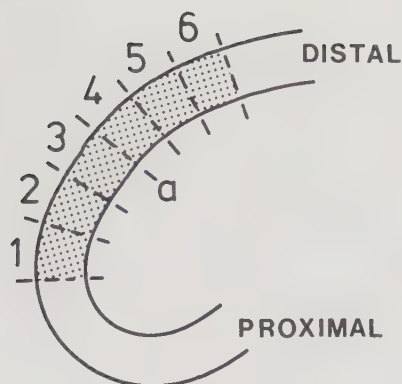


Fig. 1. Location of analyzed segments in relation to anastomosis (a).

The following terms and calculations are used in the text and diagrams:

- 1) Tissue dry weight (mg/segment)
- 2) Collagen concentration (μg hypro/mg dry weight)
- 3) Collagen content (μg hypro/segment)
- 4) Collagen synthesis, specific activity of collagen (dpm ^3H hypro/ μmol hypro)
- 5) Protein synthesis (total dpm ^3H /mg dry weight)

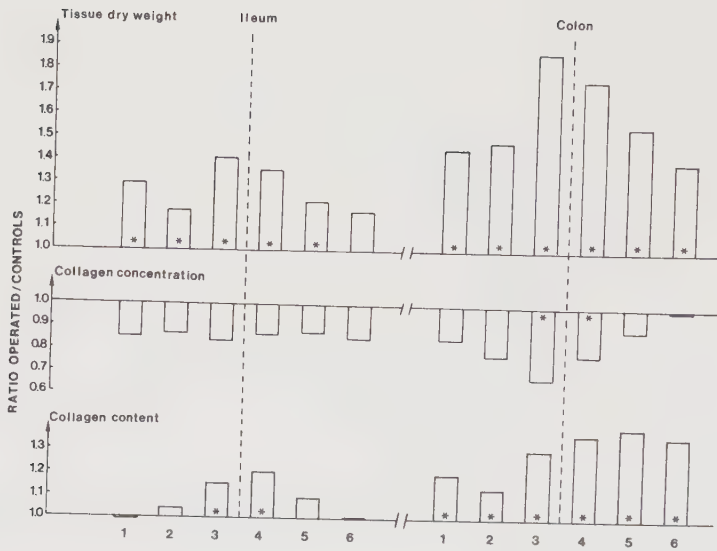
Statistical methods

Mean, standard deviation and standard error of the mean were calculated. Student's t-test for unpaired observations was used for comparisons between rats with healing anastomosis and control rats, and Student's paired t-test for intra-animal comparisons; $p < 0.05$ was considered significant.

RESULTS

Three animals with anastomotic complications were excluded from the analyses, two (dilatation) in the group with simultaneous ileum and colon anastomoses and one (abscess) on day 4 after ileum anastomosis.

DAY 2



DAY 4

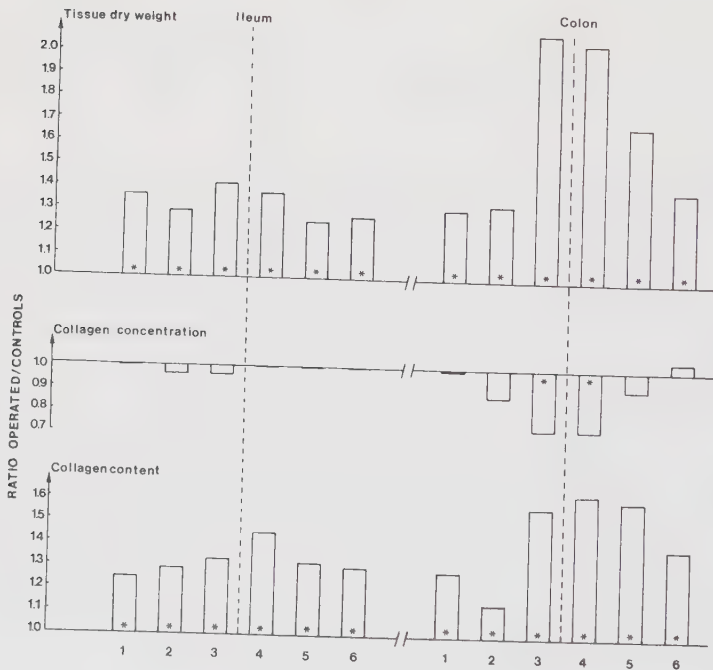


Fig. 2. Tissue dry weight, collagen concentration and collagen content in segments 1-6 (cf. Fig. 1) on days 2 and 4 after anastomosis in ileum and colon. Values expressed as ratios of rats with healing anastomoses/rats sacrificed immediately after operation (controls). * denotes significant difference from controls.

The mean body weight on the second postoperative day had fallen by 3% after ileum anastomosis and by 8% after colon anastomosis, a significant difference ($p < 0.01$). On the fourth postoperative day the respective body weight decrease was 2 and 4% ($p < 0.05$). When anastomoses were made in both ileum and colon of the same rat, the mean body weight loss on day 4 was the same as in the group with colon anastomosis only.

The tissue dry weight of the six ileum segments was increased by 20-40% during the whole period of observation. The specimen dry weight of the anastomotic segments (3+4) of the colon was increased by 75-85% and that of the other segments by 40-55% on the second day. The anastomotic segments showed further increase to day 4, when the dry weight was doubled (Fig. 2).

The collagen concentration showed no significant change in the ileum during the period of the investigation. In the colon, significant collagen decrease was observed in the biopsies close to the anastomoses on both days of investigation (Fig. 2).

The collagen content was significantly increased in the ileum on the second postoperative day in the two biopsies close to the anastomosis, but no increase was seen in the other biopsies. On day 4 a collagen increase was observed in all the ileum biopsies. In the colon, collagen content was significantly increased in all the biopsies already on the second postoperative day, with still greater rise to day 4 (Fig. 2).

Collagen synthesis on day 4 was equal in the anastomotic region of ileum and colon. In the distant biopsies of ileum (1 and 6), however, collagen synthesis was significantly greater than in the corresponding colon biopsies (Fig. 3).

Protein synthesis on day 4 was equal in small and in large intestinal biopsies (Fig. 3).

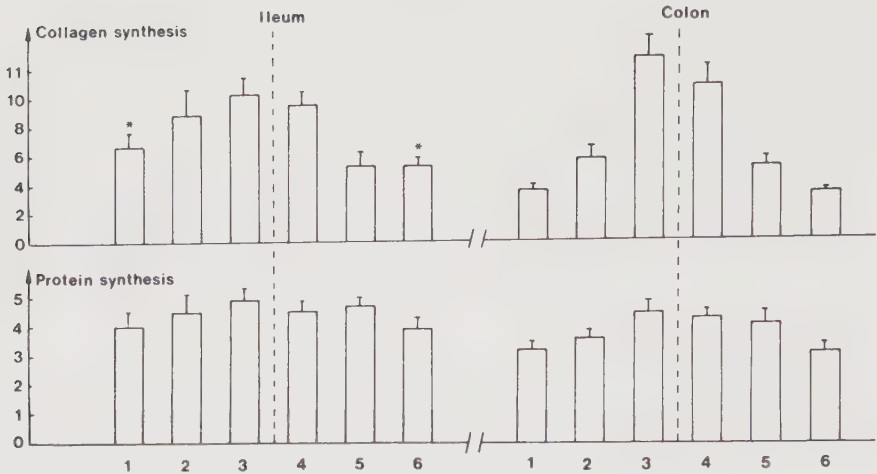


Fig. 3. Collagen synthesis expressed as specific activity (dpm ³H-hypro x 10⁵ / μmol hypro) and protein synthesis expressed as total radioactivity (total dpm ³H x 10³ / biopsy) in segments 1-6 (cf Fig. 1) on day 4 after anastomosis in ileum and colon of the same animal. Means ± SEM. * denotes significant difference between corresponding segments of ileum and colon.

DISCUSSION

The general pattern of changes in collagenous and noncollagenous compartments was similar in the small and the large intestine during the early healing of anastomoses. Differences were found, however, in the magnitude and time sequence of events.

Operations on the colon seemed to affect the rats more than ileal operations, since the weight loss was greater after colon anastomosis. When anastomosis was performed in both small and large intestine, the weight loss was the same as when only colon had been operated on.

The increase of both collagenous and noncollagenous substances occurred earlier and was more pronounced in colon than in ileum. The earlier increase in collagen content could have been due either to earlier start of collagen synthesis

in the colon or to lower degree of collagenolysis in the colon if the synthesis was the same as in ileum. The present study does not permit firm conclusions concerning the mechanism, since collagen synthesis was studied only on day 4. In the light of the observations, study of collagen synthesis already on day 2 would have been interesting. However, the later collagen accumulation in the distal segments of the ileum, combined with the finding of higher collagen synthesis in these segments on day 4, may indicate later start of collagen synthesis in ileum than in colon.

Concerning causes of the differences between colon and ileum, only speculation is possible. Intraluminal bulk in the colon, resulting in intermittent stretching, may be important, since such reactions have been shown to increase collagen and protein synthesis already after 6 hours' stimulation in muscle preparations (12). Another possibility is that the higher concentration of bacteria in the colon influences collagen and protein metabolism. Bacterial endotoxins are known to stimulate collagen synthesis in vitro (14) and in vivo (4).

Previous investigators who compared healing in small and large intestine (6,15) expressed their results only as collagen concentration. From the present study, as in others at our laboratory (1,2,11), it is obvious that collagen concentration may be misleading as regards collagen metabolism. Some findings in dogs (15) agree with ours in regard to collagen concentration, whereas in studies on rabbits there was decrease of collagen concentration both in large and in small bowel (6). Species differences may explain these differences in results.

In conclusion, weight loss in rats was more pronounced after colon anastomosis than after ileum anastomosis, indicating that the rats were more affected by the large-bowel operations. Collagen and noncollagenous substances increased earlier in colon than in ileum. The differences may have been due to greater bulk in the large intestine and/or to the higher bacterial content there than in ileum. This question will be further investigated.

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VI

HEALING OF ILEOCOLIC ANASTOMOSES

Breaking strength and collagen content in the intestinal wall
after ileocolic resection and anastomosis in the rat.

Kent Jönsson, Hasse Jiborn and Bengt Zederfeldt

VI

ABSTRACT

Breaking strength, collagen concentration and collagen content of the intestinal wall were studied in rats after ileocolic anastomosis. The values were compared with findings in control rats without operation and in previous studies of ileal and colonic anastomosis. Collagen content and concentration in the ileal wall were lower on the third day of healing than previously found during ileal healing. Breaking strength decreased in the early phase to the same extent as in previous experiments, but the restoration of strength from the third day on was more rapid than previously noted in ileal anastomoses. The changes observed in the ileum following ileocolic anastomosis were more like the pattern in colon. It is suggested that intestinal content (bacteria and intraluminal bulk) influences the collagen metabolism in the intestinal wall.

INTRODUCTION

In a previous study (12) we found that collagen content around anastomosis increased more rapidly in the left colon than in the ileum. Increase in the non-collagenous substances similarly occurred earlier and was more pronounced in left colon than in ileum. It was not possible to decide if these differences in reaction were specific for the respective parts of intestine or if they could be attributed to differences in the intestinal content, e.g. its bulk and/or bacterial content. The aim of the present study was to elucidate this question by performing an anastomosis between ileum and colon, thus eliminating differences in intestinal content. Collagen content and breaking strength of the anastomoses were determined and compared with findings in previous studies of ileal and colonic anastomoses.

MATERIAL AND METHODS

The experiments were made on 95 male Wistar rats weighing 278 ± 50 g. The rats were caged in groups of five and had access to standard laboratory diet and water at all times, with no preoperative or postoperative restrictions. They were stalled in the laboratory for at least one week before use. In 14 of the rats no anastomosis was performed, and these were used for breaking strength tests of the intact ileum. Eighty-one rats were anesthetized with choral hydrate (36 mg/100 g body weight i.p.) and the right colon to the major flexure was resected together with 5 cm of the ileum. An ileocolic anastomosis was performed using 16-17 inverting sutures of 7-0 polypropylene. Breaking strength of the anastomosis was determined in all these rats - in 14 immediately after the operation (day zero) and otherwise in groups of 11 to 24 on day 3, 4, 7 or 14. The 14 rats tested on day zero were also used for hydroxyproline analysis, as were 9 rats in the 3-day group and eight in the 7-day group. Animal weight was recorded at the beginning and at the end of the test period.

The intestine was dissected along the mesenteric border and freed from adhesions. Breaking strength was tested on the whole specimen with the sutures in place, in a specially constructed tensiometer which provided an increasing force from 0.03 to 0.05 N/s (in the interval from 0 to 3 N). The diameter of the anastomosis was measured with calipers after clamping in the tensiometer. The mean diameter was 7.4 ± 1.2 mm. Anastomotic complications were defined as dilation of the bowel (diameter > 9.5 mm), anastomotic dehiscence, and abscess or granuloma formation. Rats showing such complications were excluded from the analyses.

In the rats used for hydroxyproline analysis, the anastomoses were transected in the middle, and three consecutive 1-cm segments were cut out on either side of the transection (Fig. 1). The mucosa was scraped with scissors to remove intestinal content and mucus. The specimens were dried in an oven at 100°C to constant weight and dry weight was recor-

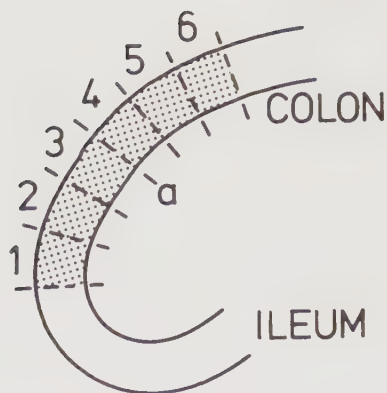


Fig. 1. Location of analyzed segments. a = anastomosis, 1,2,3 = ileal segments, 4,5,6 = colonic segments.

ded. The samples were then hydrolyzed for six hours at 130° C in 6 N hydrochloric acid and hydroxyproline (hypro) was determined according to Stegemann and Stalder (17). Collagen was studied as hydroxyproline.

Statistical methods

Mean and standard error of mean were calculated. Student's t-test for unpaired observations was used for comparisons between groups. $P < 0.05$ was considered significant.

RESULTS

Fifteen rats (22 %) with anastomotic complications were excluded.

The mean loss of body weight from the operation to the third postoperative day was 7 ± 3 %. At day 7 the operative weight was restored, and at day 14 the weight had increased by 15 ± 4 %.

Tissue dry weight (Fig. 2) was significantly increased on the third day in all three segments of ileum and in the anastomotic segment (segment 4) of colon. On day 7 a further increase of tissue dry weight was found in both anastomotic segments (3 + 4).

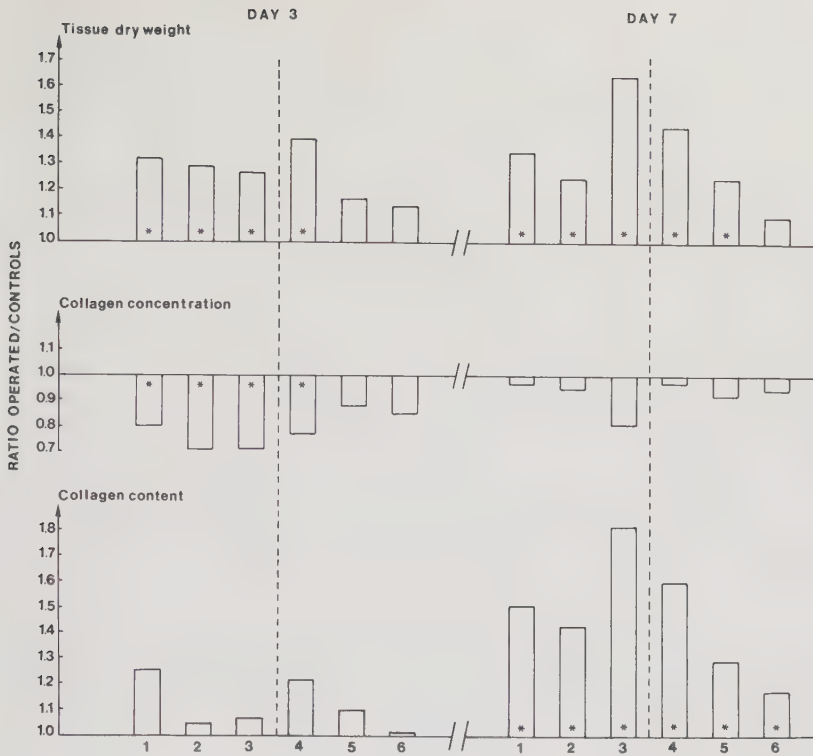


Fig. 2. Tissue dry weight (mg/segment) collagen concentration ($\mu\text{g hypro/mg tissue dry weight}$) and collagen content (mg hypro/segment) in segment 1-6 (see Fig. 1) on day 3 and day 7 after anastomosis. Values expressed as ratios: rats with healing anastomosis/controls. Broken line indicates line of anastomosis. * denotes significant difference from controls.

Collagen content (Fig. 2) was not significantly increased in any segment on the day 3, but there was significant increase in all the segments on day 7. In the anastomotic segments the rise then amounted to 60 - 80 % as compared with the controls.

Collagen concentration (Fig. 2) had decreased by 20 - 30 % on day 3 in all segments of the ileum and in the anastomotic colon segment. The values on day 7 did not differ from those in the control rats.

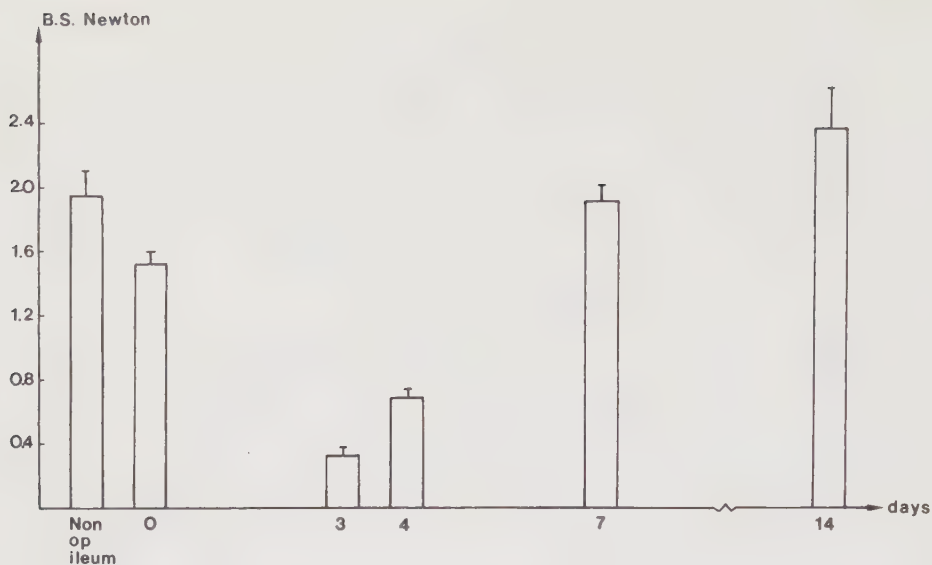


Fig. 3. Breaking strength (newtons) of ileocolic anastomosis and intact ileum (means $\pm$ SEM).

The breaking strength of ileocolic anastomoses are shown in figure 3. Three days postoperatively the anastomotic strength was 20 % of that on day zero. On day 7 the anastomoses were significantly stronger than immediately after the operation ($p < 0.01$) and were equal to the strength of intact ileum. In 40 % of the tests the break occurred outside of the anastomosis. On day 14 the break took place proximal to the anastomosis in 80 % of the tests.

DISCUSSION

In a previous study (12) differences between ileal and colonic anastomoses were found as regards accumulation of collagen and non-collagenous substances. The present study was undertaken to evaluate the reasons for these differences. Collagen content and development of strength were studied in ileocolic anastomoses. In such anastomoses the ileum is exposed to the same intestinal content as in colon. Since our interest was focused on possible changes in the ileum as

a consequence of this exposure, we shall discuss only the ileum side as regards collagen and non-collagenous substances.

The collagen content on the ileum side of ileocolic anastomosis on the third postoperative day was found to be less than was previously observed after ileo-ileal anastomoses (12,14). The increase of tissue dry weight, however, was of the same magnitude as previously noted in ileo-ileal anastomoses (12,14). These changes resulted in decreased collagen concentration in the ileal segments of the ileocolic anastomoses. Such a decrease was not observed during any phase of healing of ileo-ileal anastomoses in rats (12,13,14) or in dogs (15,20). The decreased collagen concentration, on the other hand, was similar to earlier findings in left colon anastomosis (3,8,10,12). In ileocolic anastomosis, therefore, the anastomosed ileum assumed the healing pattern in the colon. This strongly indicates that the intestinal content influences collagen in the intestinal wall around an anastomosis. Since collagen synthesis was not determined in this investigation, it was not possible to conclude if the lower content of collagen was attributable to depression of collagen synthesis or to increase of collagen lysis.

The diminution of breaking strength to the third day in ileocolic anastomosis was identical with the decrease observed in ileo-ileal anastomosis (11). The restoration of anastomotic strength, however, was more rapid in ileocolic than in ileo-ileal anastomosis (11). Already on day 7 the anastomotic strength of ileocolic anastomosis, tested with sutures in place, surpassed the strength of newly performed anastomosis (day zero) and approached the strength of intact ileum. In 40 per cent of the tests the break occurred outside the anastomoses, a phenomenon not observed until day 28 in ileo-ileal anastomoses.

Concerning the causes of the increased rate of gain in anastomotic strength, only speculation is possible. Conceivable mediators are differences in intestinal content, e.g. in-

creased numbers of bacteria (5) or heightened tension due to intraluminal bulk (1). The bacterial numbers seem to be the more plausible factor, since there is scarcely any difference in intraluminal bulk between ileo-ileal and ileocolic anastomoses. The role of bacteria is supported by observations on infected colonic anastomoses (7,9) and on healing of colonic anastomoses after resection for experimental "diverticulitis" (21).

Other studies showed that the strength of skin wounds is increased by limited contamination with bacteria (6,16,18). Both bacterial type (18) and concentration (16) seem to be important for this reaction. Bacterial endotoxin has further been shown to stimulate collagen synthesis in vivo (4) and in vitro by macrophage release of fibroblast-activating factors (19).

It is plausible that heightened bacterial numbers in and around an ileocolic anastomosis both increases lysis in the intestinal wall and stimulate collagen formation in the anastomotic line, leading to increased breaking strength on the seventh postoperative day. Two observations made in the studies of colonic anastomoses lend some support to this view. Firstly, anastomoses in the colon showed an earlier and faster gain of breaking strength than did ileo-ileal anastomoses (1,8). Secondly, diversion of the fecal stream by a proximal colostomy resulted in reduction of the strength of colonic anastomoses, which could have been due to diminished bacterial content (2).

In summary, in rats with ileocolic anastomosis, the anastomosed ileum assumed the healing pattern of the colon. The findings indicate that the intestinal content, and most probably its bacterial density, influence the collagen in the intestinal wall as regards both collagen accumulation and development of anastomotic strength.

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